

Research Article

Effects of Increasing Indoor Negative Air Ions on Cognitive Performance and Health of High Pure CO₂ Level-Exposed College Students

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Cognitive performance is essential to foster learning. High CO₂ concentrations are common in classrooms and can lead to reduced cognitive performance. Negative air ions (NAIs) can improve cognitive performance. This study explored the effects of indoor NAIs on the cognitive performance and health of college students exposed to a high pure CO₂ environment. Forty college students were exposed to four sets of conditions (NAIs+500 ppm CO₂, 500 ppm CO₂, NAIs+2500 ppm CO₂, and 2500 ppm CO₂). Participants' cognitive performance, including reasoning, short-term memory, concentration, and verbal ability, was assessed under each condition using the Cambridge Brain Sciences tool. Acute health symptoms were investigated using a subjective questionnaire, and simultaneously, participants' blood pressure, heart rate, and lung function were tested. Analysis of variance (ANOVA) in a repeated-measures design was used to analyze the effects of different conditions on cognitive performance and health symptoms. The results revealed that the different levels of CO₂ and NAIs had a significant effect on cognitive performance after one hour of exposure and had no significant effect after three hours of exposure. Compared with 500 ppm CO₂, 500 ppm CO₂+NAIs resulted in better reasoning skills, short-term memory, and verbal skills, and 2500 ppm CO₂ led to poorer reasoning skills. The addition of NAIs to 2500 ppm CO₂ improved reasoning skills, short-term memory, and verbal skills. The benefits of adding NAIs to high pure CO₂ condition on cognitive performance are more noticeable than those to low CO₂ condition. Moreover, adding NAIs can reduce nasal irritation or dryness, skin irritation or dryness, sleepiness symptoms, and heart rate elevation caused by pure CO₂. However, the benefits of NAIs on health symptoms and physiology were not observed under the 500 ppm CO₂ condition. Adding NAIs to a high pure CO₂ level is an effective means to improve the cognitive performance and health of college students.

1. Introduction

Indoor air quality (IAQ) affects productivity [1], mood [2], cognitive performance [3], and health [4] of individuals. CO₂ is an important indicator of IAQ. Existing guidelines for indoor CO₂ stipulate that CO₂ concentrations ≤ 1000 ppm represent good or excellent IAQ, 1000–1500 ppm represent acceptable or moderate IAQ, and concentrations > 1500 ppm represent poor IAQ [5]. Humans are the main source of indoor CO₂; therefore, indoor CO₂ levels depend

mainly on human occupancy and air exchange rate. CO₂ concentrations in schools may exceed 2000 ppm with increased room occupancy and reduced building ventilation [6]. A study in Albania found that the average weekly CO₂ levels during classes ranged from 1286 to 5546 ppm [7]. The CO₂ concentration in enclosed air-conditioned classrooms is generally greater than 2500 ppm and occasionally reaches 3500 ppm [8].

Both exposures to pure CO₂ and CO₂ emitted by people affect cognitive performance and health. Maula et al. [9]

reported that the results of information retrieval and an operation span task were negative in a space with a CO₂ concentration of 2260 ppm emitted by people. Furthermore, field measurements in schools reported an evident correlation between CO₂ concentration and reduced concentration [10]. By analyzing the influence of different ventilation rates on indoor CO₂ concentration in the classroom, it is found that reducing the CO₂ concentration in classrooms from 2100 to 900 ppm would increase performance speed by 12% and accuracy by 2%. Reducing the CO₂ concentration from 2400 ppm to 900 ppm would improve the performance of national tests and school-leaving examinations by 5%, and reducing CO₂ from 4200 ppm to 1000 ppm would increase children's daily attendance by 2.5% [11]. Twenty-two participants were exposed to pure CO₂ at 600, 1000, and 2500 ppm in an office-like chamber, and statistically significant decrements occurred in cognitive performance (decision-making, problem resolution) starting at 1000 ppm [12]. High levels of CO₂ exposure can also cause respiratory problems. According to research conducted in schools, the onset of daytime breathlessness is associated with a CO₂ concentration of 1208 ppm [13], and children exposed to indoor CO₂ concentrations above 1000 ppm develop respiratory symptoms [14]. The effects of increased CO₂ concentration on neurological symptoms and irritation of the upper airway system have been investigated; a 100 ppm increase in CO₂ is strongly associated with both neurological symptoms and irritation [15]. A study conducted on the United Arab Emirates University campus reported that high CO₂ levels may lead to headaches [16]. Increased CO₂ levels also harm the physical responses of occupants; when the CO₂ level increased to 2700 ppm, heart rate and sleepiness increased [17]. Diastolic blood pressure and salivary α -amylase levels were significantly increased when the metabolically generated CO₂ level was 3000 ppm [18].

Negative air ions (NAIs) are produced by plants, shear forces of water, sunlight, atmospheric radiant or cosmic rays, and natural and artificial corona discharges [19]. NAIs are less prevalent in urban environments than in forests, places with flowing water, or mountainous areas [20]. Currently, NAI generators are widely used for domestic and industrial purposes because they reduce aerosol particles, odors, airborne microorganisms, and volatile organic compounds in indoor air [21]. Previous speculations suggest that exposure to NAIs may be associated with beneficial effects on cognitive performance and health [19, 22]. A study exploring the effect of NAIs on cognitive performance among young male adults showed that people exposed to a NAI environment had shorter reaction times and higher accuracy in both Stroop congruent and incongruent trials [23]. Wallner et al. reported that short-term exposure of nonsmoking volunteers to high NAI concentrations improved cognitive performance related to verbal, reasoning, and perceptual speed skills [24]. Furthermore, Arora et al. revealed that the selective and sustained attention of adolescents can be enhanced following NAI therapy [25]. In addition, NAIs affect the cardiovascular system. Zhang et al. reported that exercising with NAIs significantly improved blood pressure, heart rate, ventilation volume, and hemoglobin levels [26]. However, artificially

produced NAIs may deteriorate heart rate variability and lower energy generation in children. The mechanism by which NAIs affect cardiovascular health remains unclear.

Currently, high CO₂ levels in classrooms are difficult to reduce within a short period, which can lead to negative effects on students' cognitive performance and health. Previous studies have explored the benefits of adding NAIs to a normal environment on cognitive performance. However, no studies have explored the benefits of NAIs in the context of high CO₂ concentrations. It is unclear whether NAIs can be introduced in classrooms as a positive factor in improving the adverse effects of CO₂ on college students' cognitive performance and health. Our study investigated the effects of NAIs on the cognitive performance and health symptoms of college students exposed to high CO₂ levels by setting up four conditions (NAIs+500 ppm CO₂, 500 ppm CO₂, NAIs+2500 ppm CO₂, and 2500 ppm CO₂) to fill this research gap. Cognitive performance is a complex process that requires students to use and apply a range of cognitive skills including memory, reasoning, concentration, and verbal ability [27]. Here, the generic cognitive skills underlying the learning processes were measured. Cognitive performance was measured after one and three hours of exposure to the different conditions. We investigated the intensity of acute health symptoms in the participants after three hours of exposure to the different conditions using a questionnaire. Simultaneously, blood pressure, heart rate, and lung function of the participants were tested to assess their cardiopulmonary health.

2. Material and Methods

2.1. Participants and Experimental Conditions. The participants were exposed in a chamber to different CO₂ and NAI levels and were blind to conditions. During the exposures, cognitive performance tests were conducted twice. After the exposures, they reported their acute health symptoms, and physiological measurements were measured. The experiment was conducted in two identical adjacent chambers ($L \times W \times H = 5.8 \text{ m} \times 5.0 \text{ m} \times 2.8 \text{ m}$). The indoor temperature was controlled by an air conditioner. Previous findings suggest an inverted U-shaped relationship between cognitive performance and indoor air temperature within the 20–32°C range, with the highest performance peaking at 21.6°C [28]. Therefore, indoor temperature was maintained at 22°C during the test. The typical indoor CO₂ concentration tested in school buildings is 2000–3000 ppm [29]; thus, 2500 ppm was set as the high CO₂ level in this study. The indoor CO₂ concentration under natural ventilation was selected as the low CO₂ level. The low CO₂ concentration was approximately 500 ppm, which is similar to the CO₂ concentration in the outside air and is at a comfortable level [30]. High CO₂ level was achieved by a combination of pure CO₂ release and the accumulation of CO₂ emissions from the participants by closing the windows and doors. Before the participants entered the laboratory, pure CO₂ was released into the room through the ducts until the CO₂ concentration reached 2500 ppm. A pedestal fan was used to achieve rapid mixing [17]. After the participants

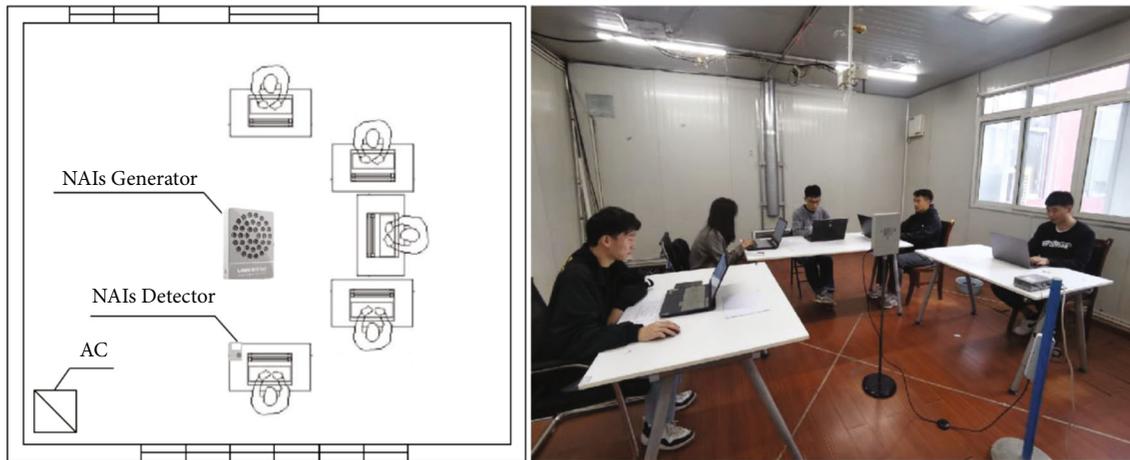


FIGURE 1: The layout of the experimental room.

entered the room, the CO_2 emitted by them was allowed to accumulate by closing the doors and windows. Owing to air infiltration, indoor CO_2 escapes; thus, when the indoor CO_2 concentration decreases below 2500 ppm, pure CO_2 from the cylinder is released into the room, resulting in a CO_2 concentration of 2500 ppm. NAIs were released through an NAI generator (ZS-A8, Zuoshan Industrial Group Co., Ltd., China), which generates NAIs without producing ozone. The NAI generator was located 1.8 m away from each participant's seat. When the NAI generator was turned on, the NAI concentration in the subject's breathing zone was approximately 9×10^4 ion/cm³, which is similar to the NAI concentration in previous studies regarding the effects of NAIs on cognitive performance [31, 32]. Here, this level of NAIs was used as the high level. The NAI concentration in the room with the NAI generator turned off was considered a low level. Whether the NAI generator is turned on or not, the location of the NAI generator is not changed, and when the NAI generator is turned on, only the indicator light is on, and there is no noise. The lights were blocked during the experiment; therefore, the participants were unaware of the environmental conditions. The four conditions used in this study were C1: NAIs+500 ppm CO_2 , C2: 500 ppm CO_2 , C3: NAIs+2500 ppm CO_2 , and C4: 2500 ppm CO_2 . The experiment was conducted simultaneously in two offices with five participants per chamber. The participants' tables were distributed around the NAI generator to ensure that the NAI exposure was distributed equally (Figure 1).

To determine the minimum sample size required to satisfy the experimental requirements, statistical efficacy calculations were performed using the G*Power software (Heinrich-Heine-Universität, Düsseldorf, Germany). A within-subject design with repeated measures was used in this study. Therefore, the a priori statistical efficacy calculation method of repeated-measures ANOVA was used to determine the sample size required for the experiment. Based on a previous study [33], the effect size f can be taken as 0.4, the correlation among measures to be 0.5 by default, a nonsphericity correction to be 1, a statistical power ($1 - \beta$ err prob) to be 0.8 in general, and the significance level (α

err prob) was taken as 0.05. The minimum sample size required for this experiment was calculated to be 36. Therefore, 40 college students were recruited to ensure a balanced design and meet the requirements of the minimum sample size by advertising at Chongqing University. There were 21 females and 19 males with a mean age of 23 ± 0.2 (mean \pm standard deviation (SD)) years and a mean body mass index of 20.5 ± 0.4 kg/m². All participants were healthy and did not smoke. They were paid CNY 30 per hour for taking part in the experiments. The Ethics Review Committee of Life Sciences at Central China Normal University approved the study method. Ethics Ratification ID is CCNU-IRB-2019-002. All subjects provided written informed consent to participate in the experiments.

2.2. Measurements

2.2.1. Physical Measurements. The indoor temperature, relative humidity, NAIs, CO_2 , and particulate matter (PM_{2.5}) concentrations were determined. The indoor temperature, humidity, and CO_2 concentration were measured using an air quality monitor (BR-A, BRAMC Medical & Technology Co., Ltd. China). NAI concentrations were determined using an NAI monitor (WST-08 Beijing Vostong Technology Co., Ltd. China). The measurement range and accuracy of the instruments for the different parameters are listed in Table 1. To make the values close to the participants' environmental exposure levels, the air quality and NAI monitors were placed on the participants' tables at a height of approximately 1.2 m, covering the breathing zone of the participants. The environmental parameters were monitored during the experiments. All monitors were calibrated before the experiment to ensure data quality.

2.2.2. Subjective Measurements. The intensity of acute health symptoms was measured using a questionnaire. A survey of acute health symptoms included eye irritation or dryness, weeping, throat irritation or dryness, desire to cough, wheezing, nasal irritation or dryness, runny nose, skin irritation or dryness, sleepiness, headache, and tingling of the extremities

TABLE 1: Major specifications of the measuring instruments in this experiment.

Parameter	Range	Accuracy
Air temperature	-40°C ~60°C	±0.5°C
Relative humidity	20%~95%	±3%
CO ₂	0~5000 ppm	±15%
NAIs	0 ~ 5 × 10 ⁶ ion/cm ³	±5%
PM _{2.5}	0~999 µg/m ³	±15%

Right now I feel as follows:

Eyes irritation or dryness	_____
Weeping	_____
Throat irritation or dryness	_____
Desire to cough	_____
Wheezing	_____
Nasal irritation or dryness	_____
Runny nose	_____
Skin irritation or dryness	_____
Sleepiness	_____
Headache	_____
Tingling of the extremities	_____

FIGURE 2: The questionnaire of the acute health symptoms. The leftmost side in every line means no symptom (scored as 0), from left to right the symptom worsens, and the rightmost side in every line means the symptom is very serious (scored as 100).

[34, 35]. At the end of the exposure, the participants recorded their feelings with a pen on linear visual analog rating scales, which are often used to describe acute health symptoms [33]. The test sheet is shown in Figure 2.

2.2.3. Cognitive Performance. Short-term memory, reasoning, concentration, and verbal skills were tested using online cognitive performance tests from the public website of Cambridge Brain Sciences (CBS) developed by Cambridge University [36]. The CBS suite of neurocognitive tasks is derived from traditional pen-and-paper tests and accurately measures the core elements of cognition, such as short-term memory, reasoning, attention, and verbal ability. These tests have been validated through decades of scientific research [12, 37, 38]. Twelve separate tests were included in the CBS tool, and the participants were asked to complete all 12 tests. The Odd One Out, Polygons, Rotations, and Spatial Planning tasks reflect reasoning skills. Odd One Out assesses deductive reasoning and requires reasoning regarding the features of several shapes to deduce a shape that does not fit the rest. Polygons assess visuospatial processing by challenging the patients' proficiency in identifying subtle differences between shapes. Rotation is a function of the visual representation of the brain. Spatial Planning assesses a patient's ability to act with forethought and sequential behavior to achieve specific goals. The Monkey Ladder, Paired Associates, Spatial Span, and Token Search tasks reflect the short-term memory. The Monkey Ladder assesses

TABLE 2: Experimental sequence balanced with Latin square balanced design.

	1 st exposure	2 nd exposure	3 rd exposure	4 th exposure
Group 1	C2	C1	C3	C4
Group 2	C1	C4	C2	C3
Group 3	C3	C2	C4	C1
Group 4	C4	C3	C1	C2

visuospatial working memory, requires storing numbers and their locations, and then translates it into a series of movements in space. Paired Associates assesses episodic memory by asking patients to remember which objects they previously saw, along with the location where they were seen. The Spatial Span is a cognitive system that allows for the temporary storage of spatial information. During Token Search, patients must maintain and update an ongoing representation of previous searches in a self-directed task. Double Trouble and Feature Match tasks reflect concentration skills. Double Trouble (based on the Stroop task) assesses response inhibition, which is the ability to concentrate on relevant information to make an appropriate response even when distracting information or interference is present. Feature Match assesses the ability to muster mental resources to focus and monitor a specific stimulus or difference. The Digit Span and Grammatical Reasoning tasks reflect verbal skills by measuring verbal short-term memory, defined as a system that allows for the temporary storage of information and is crucial in everyday tasks, such as remembering a telephone number or understanding long sentences. Digit Span involves numbers, but performance is indicative of verbal short-term memory because it requires dealing with items in a specific order, as opposed to spatial short-term memory. Grammatical Reasoning determines the ability to quickly understand and draw valid conclusions about concepts expressed in words by understanding complex sentences with multiple negative statements.

2.2.4. Physiological Measurement. Cardiorespiratory functions including blood pressure, heart rate, and lung function (forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), and peak expiratory flow (PEF)) were measured. Blood pressure and heart rate were measured using an upper-arm electronic sphygmomanometer (U15; OMRON, China). Lung function was assessed by using a spirometer (A01; Breath Home, China).

2.3. Experimental Protocol. This study assessed the effects of four different conditions (C1: NAIs+500 ppm CO₂, C2: 500 ppm CO₂, C3: NAIs+2500 ppm CO₂, and C4: 2500 ppm CO₂) on cognitive performance, the intensity of acute health symptoms, and physiological measurements of college students. A within-subject design was used in this experiment. In the within-subject design, all participants were exposed to each condition. Therefore, individual differences can be offset more efficiently. Participants are likely to score better on cognitive tests in the latter than in the previous test, which is

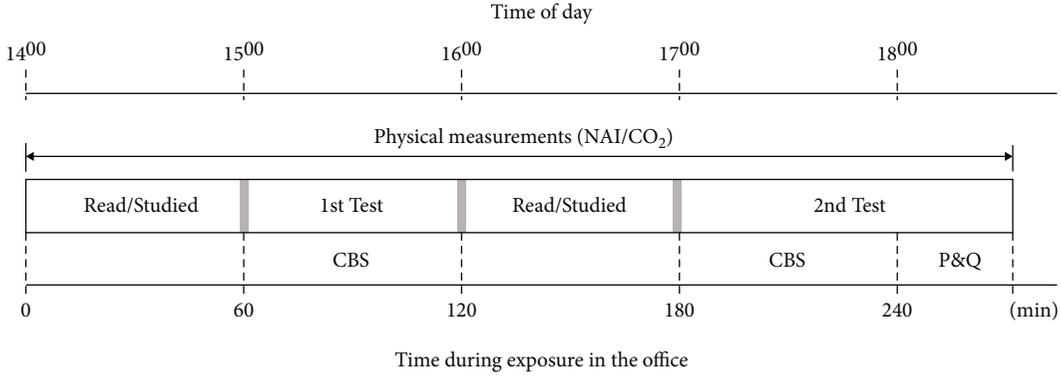


FIGURE 3: Schedule of events taking place during each exposure. P&Q: physiological test and questionnaire.

recognized as a learning effect. Therefore, the 40 participants were equally divided into four subgroups of 10. A balanced 4×4 Latin square design was used to control for the learning effect, as shown in Table 2. Each exposure lasted for 4 h and was conducted from 14:00 to 18:00. During each exposure, participants underwent cognitive performance tests twice to assess the effect of exposure length on cognitive performance. The schedule for each exposure is shown in Figure 3. At 14:00, the participants entered the office. From 14:00 to 15:00, participants adapted to the indoor environment, during which time they read or studied. From 15:00 to 16:00, the participants spent approximately 60 min on their laptops to complete the 12 CBS cognitive tests. From 16:00 to 17:00, the participants read or studied. From 17:00 to 18:00, the participants spent approximately 60 min completing the 12 CBS cognitive tests. Upon completion, the participants were asked to complete an acute health symptom questionnaire, and their physiological parameters were measured.

Cognitive tests were conducted twice after 1 and 3 h of exposure, respectively, because 1 and 3 h are the durations for short- and long-term exposure of college students indoors [39]. A week preceding the first exposure session, all participants attended a 2 h induction session to familiarize themselves with the experimental procedure and to receive training and practice on questionnaires and online cognitive tests. After the induction session, the participants were asked to practice each of the 12 cognitive tests five times before the experiment to avoid the learning effect. The participants always experienced the four conditions on the same day of the week for four successive weeks. The participants were asked to rest well the night before each exposure and not to wear any strong perfumes during the experiment. Water and bread were provided to relieve participants' fatigue and hunger during the experiment. All participants were healthy, did not take any medication during the experiment, and provided written informed consent before participation.

2.4. Statistical Analysis. The scores on each of the 12 cognitive tests were notably different. Therefore, to compare test scores between different participants and cognitive tests, each participant's score was standardized using the average score of the same person on a particular cognitive

test. Equation (1) was used to standardize the test data for each participant:

$$s(x_{i,j}) = \frac{x_{i,j}}{(1/n)\sum_{j=1}^n x_{i,j}} \times 100. \quad (1)$$

In Equation (1), $x_{i,j}$ refers to the performance of the i^{th} participant under condition j and n refers to the total number of conditions.

First, the Mann–Whitney U test was used to determine whether there were significant differences in the environmental parameters under the four exposure conditions. The results of the cognitive tests, acute health symptoms, and physiological measurements were tested for normality using the Shapiro–Wilk's W test, with the significance level set at $P < 0.05$. Normally distributed data were subjected to analysis of variance in a repeated-measures design with each subject as his own control, thus excluding any differences in experience, training, or intellectual skills that can influence performance. In repeated-measures analysis of variance, the within-subject factor is defined as the conditions (4) and time (2). Huynh–Feldt statistics and Greenhouse–Geisser statistics were used to adjust for the violation of sphericity. When exposure conditions had a significant effect on the results of cognitive tests, acute health symptoms, and physiological measurements, post hoc analyses (Tukey's HSD test) were used to compare differences between different exposure conditions. All statistical tests were 2-sided with $\alpha = 0.05$, and all statistical analyses were performed using SPSS version 26 (IBM Inc., USA).

3. Results

3.1. IAQ Parameters. The indoor air parameters for the four conditions are listed in Table 3. The concentrations of CO_2 and NAIs did not deviate from the intended level. The low CO_2 level was close to 500 ppm, and the high CO_2 level was approximately 2500 ppm. According to the Mann–Whitney U test, there was no significant difference in the CO_2 concentration between C1 and C2 and between C3 and C4. The low NAI level was approximately 70 ion/cm^3 , and the high NAI level was approximately $9.5 \times 10^4 \text{ ion/cm}^3$. According to the Mann–Whitney U test, there was no

TABLE 3: Mean values \pm standard deviation (SD) of indoor air parameters of four conditions.

Parameters	C1	C2	C3	C4
Air temperature ($^{\circ}$ C)	21.7 \pm 0.5	21.6 \pm 0.5	21.5 \pm 0.6	21.6 \pm 0.8
Relative humidity (%)	35.1 \pm 5.1	33.1 \pm 3.3	37.0 \pm 4.3	38.9 \pm 5.5
NAIs (ion/cm ³)	94876.2 \pm 1857.1	65.8 \pm 5.7	95891.2 \pm 1635.4	70.9 \pm 6.3
CO ₂ (ppm)	488.4 \pm 38.2	470.5 \pm 52.4	2520.0 \pm 59.9	2523.7 \pm 39.0
PM _{2.5} (μ g/m ³)	9.1 \pm 3.8	32.4 \pm 4.4	10.9 \pm 4.3	37.5 \pm 4.5

TABLE 4: The mean \pm SD of 12 cognitive tests under four conditions.

	Cognitive tests	C1	C2	C3	C4
Reasoning skill	Odd One Out	13.96 \pm 1.87	12.81 \pm 2.45	13.76 \pm 1.96	13.25 \pm 2.62
	Polygons	67.68 \pm 22.48	68.28 \pm 27.5	69.90 \pm 23.00	66.00 \pm 23.06
	Rotations	132.73 \pm 46.08	126.89 \pm 38.03	132.24 \pm 45.41	116.33 \pm 46.34
	Spatial Planning	54.10 \pm 19.22	52.24 \pm 16.48	54.08 \pm 17.88	52.21 \pm 16.42
Short-term memory skill	Monkey Ladder	8.79 \pm 1.56	8.78 \pm 1.33	9.06 \pm 1.21	8.76 \pm 1.27
	Paired Associates	5.79 \pm 1.16	5.69 \pm 1.07	5.94 \pm 1.19	5.71 \pm 0.87
	Spatial Span	6.68 \pm 1.05	6.46 \pm 0.92	6.80 \pm 1.11	6.69 \pm 1.02
	Token Search	9.63 \pm 2.51	9.23 \pm 2.10	9.63 \pm 2.55	8.95 \pm 2.06
Concentration skill	Double Trouble	61.73 \pm 14.38	60.53 \pm 13.33	61.60 \pm 11.71	60.86 \pm 11.91
	Feature Match	164.09 \pm 34.01	155.98 \pm 36.47	163.70 \pm 33.5	158.88 \pm 41.73
Verbal skill	Digit Span	9.95 \pm 1.60	9.45 \pm 1.63	9.98 \pm 1.49	9.55 \pm 1.60
	Grammatical Reasoning	19.61 \pm 5.32	19.59 \pm 5.16	19.99 \pm 5.10	18.51 \pm 5.29

significant difference in the NAI concentration between C1 and C3 and between C2 and C4. The PM concentration is about 10 μ g/m³ with NAIs (C1 and C3), while the PM concentration is over 30 μ g/m³ with no NAIs (C2 and C4). NAIs can significantly reduce indoor PM concentrations. Furthermore, there were no large differences in the air temperature and relative humidity among the four conditions.

3.2. Cognitive Performance Results. The means and SD of the 12 cognitive tests exposed to the four conditions are listed in Table 4. In all 12 cognitive performance tests, college students scored much higher than the corresponding general benchmark results for all CBS website users [40].

The cognitive test results, which were significantly affected by the indoor conditions, are shown in Figure 4 and estimated marginal means of cognitive tests that have no significant association with exposure conditions are shown in Figure S1. The different levels of CO₂ and NAI conditions indoors had a significant effect on the cognitive performance only for the first test (1 h of exposure) and no significant effect on any cognitive test performance for the second test (3 h of exposure). In the first test, it was found that different conditions significantly affected the performance of Odd One Out and Rotations in reasoning skill, Token Search and Spatial Span in short-term memory, and Grammatical Reasoning and Digit Span in verbal skill; however, there was no significant effect on the performance of tests in concentration skill. Adding NAIs to 500 ppm

CO₂ resulted in better performance in reasoning and verbal skills. Compared with 500 ppm CO₂, 500 ppm CO₂+NAIs resulted in an increase in the estimated marginal means of Odd One Out, Spatial Span, and Digit Span by 11.41, 6.72, and 8.34, respectively. Higher CO₂ exposure leads to poorer reasoning skills. Exposure to 2500 ppm CO₂ resulted in a significant decrease in the estimated marginal mean of Rotations 13.57 compared with 500 ppm CO₂. The addition of NAIs at 2500 ppm CO₂ also improved the reasoning, short-term memory, and verbal skills of college students. Compared with exposure to 2500 ppm CO₂, NAIs+2500 ppm CO₂ resulted in significantly increased estimated marginal means of Rotations, Token Search, Spatial Span, Grammatical Reasoning, and Digit Span of 18.57, 11.63, 4.60, 12.56, and 5.92, respectively. Additionally, all cognitive test scores were similar between the 500 ppm CO₂+NAIs and 2500 ppm CO₂+NAI groups. The performance of reasoning, short-term memory, and verbal skills was better in the 2500 ppm CO₂+NAI condition than in the 500 ppm CO₂ condition. The estimated marginal mean of Odd One Out, Token Search, Spatial Span, and Digit Span in the NAIs+2500 ppm CO₂ environment was 10.35, 7.48, 7.57, and 7.65 higher than that in the 500 ppm CO₂ condition. By and large, compared with exposure to 500 ppm CO₂, exposure to 2500 ppm CO₂ significantly reduced the score of Rotations. Adding NAIs to 500 ppm CO₂ will significantly increase the scores of Odd One Out, Spatial Span, and Digit Span.

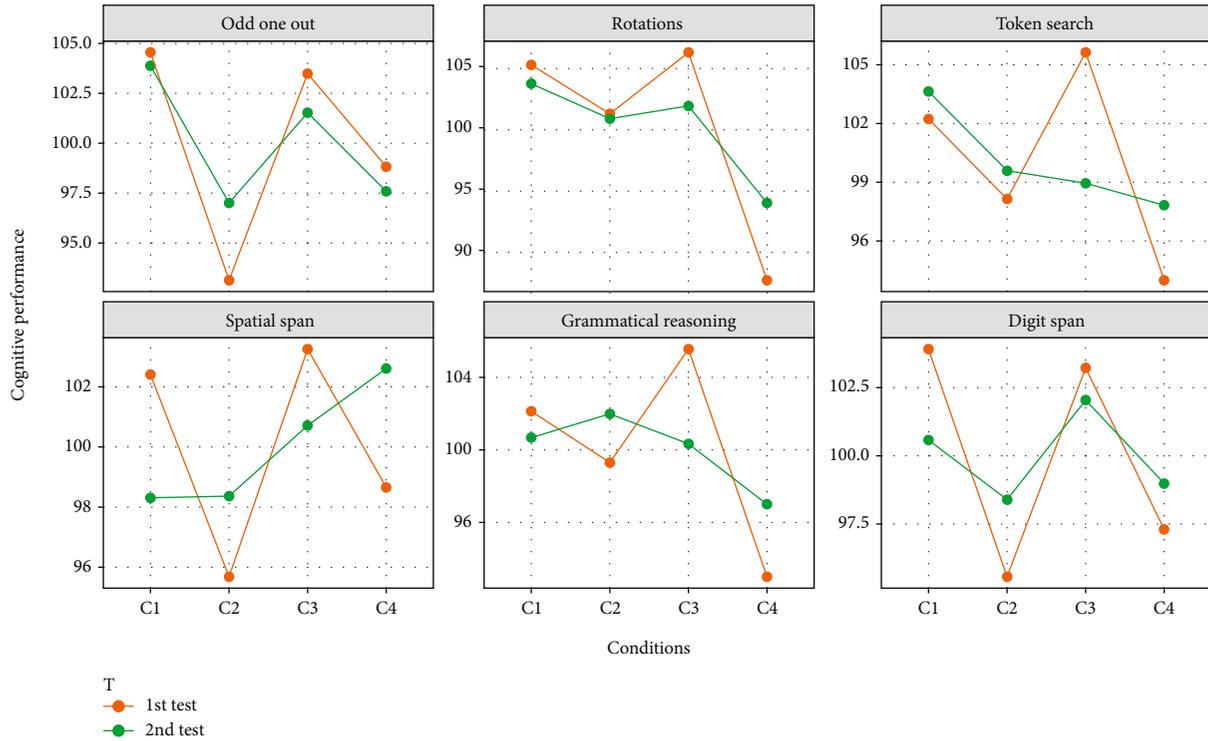


FIGURE 4: Estimated marginal means of cognitive tests that have significant association with exposure conditions (C1: NAIs+500 ppm CO₂, C2: 500 ppm CO₂, C3: NAIs+2500 ppm CO₂, and C4: 2500 ppm CO₂).

Compared with exposure to 2500 ppm CO₂, adding NAIs to 2500 ppm CO₂ will significantly improve the performance of Rotations, Token Search, Spatial Span, Grammatical Reasoning, and Digit Span. Therefore, the addition of NAIs to 2500 ppm CO₂ would have a greater benefit on the cognitive performance of college students than 500 ppm CO₂. We also found that most cognitive performance scores were higher in the second test than in the first test under the 500 ppm CO₂ and 2500 ppm CO₂ conditions. However, compared to the first test, the cognitive performance scores in the two conditions with NAIs were slightly lower in the second test, possibly due to the ability of NAIs to improve cognition decreased over time.

3.3. Health Symptoms and Physiology Results. Figure 5 shows the intensity of the acute health symptoms that have a significant association with exposure conditions, and the results of acute health symptoms that have no significant association with exposure conditions are shown in Figure S2. Elevated indoor CO₂ levels increased the intensity of nasal irritation or dryness and skin irritation or dryness symptoms reported by college students. The estimated marginal means of nasal irritation or dryness and skin irritation or dryness significantly increased by 14.1 and 9.9, respectively, in the 2500 ppm CO₂ condition compared with those in the 500 ppm CO₂ condition. Adding NAIs to 2500 ppm CO₂ condition can improve acute health symptoms in college students. Exposure to NAIs+2500 ppm CO₂ for 4h significantly reduced the estimated marginal means of nasal irritation or dryness, skin irritation or dryness, and sleepiness by 9.58, 10.5, and 16.4 compared

with 2500 ppm CO₂ condition. However, the benefits of NAIs on acute health symptoms were not observed under 500 ppm CO₂ conditions. There was no significant difference in acute symptom scores between the 500 ppm CO₂ and 500 ppm CO₂+NAI conditions. Participants exposed to NAIs+500 ppm CO₂ had essentially the same scores for acute health symptoms as those exposed to NAIs+2500 ppm.

Among the physiological outcomes, no changes in blood pressure or lung function were related to the exposure conditions, and only heart rate was related to the conditions after 4 h of exposure. Figure S3 shows the estimated marginal means and the 95% CI of blood pressure and lung function. Figure 6 shows the estimated marginal means and the 95% CI of heart rate under the four conditions. In the 2500 ppm CO₂ condition, the estimated marginal mean of heart rate was 3.18 bpm higher than that in the 500 ppm CO₂ condition. Adding NAIs to high CO₂ condition can prevent this increase. The estimated marginal mean of heart rate in the NAIs+2500 ppm CO₂ condition was reduced by 3.27 compared to the 2500 ppm CO₂ condition. The benefit of NAIs on heart rate reduction was not observed under the 500 ppm CO₂ condition. The participant's heart rates were similar among NAIs+500 ppm CO₂, 500 ppm CO₂, and NAIs+2500 ppm CO₂ conditions.

4. Discussion

Our experimental results suggest that exposure to pure 2500 ppm CO₂ is associated with a decrease in overall cognitive performance in college students compared to 500 ppm

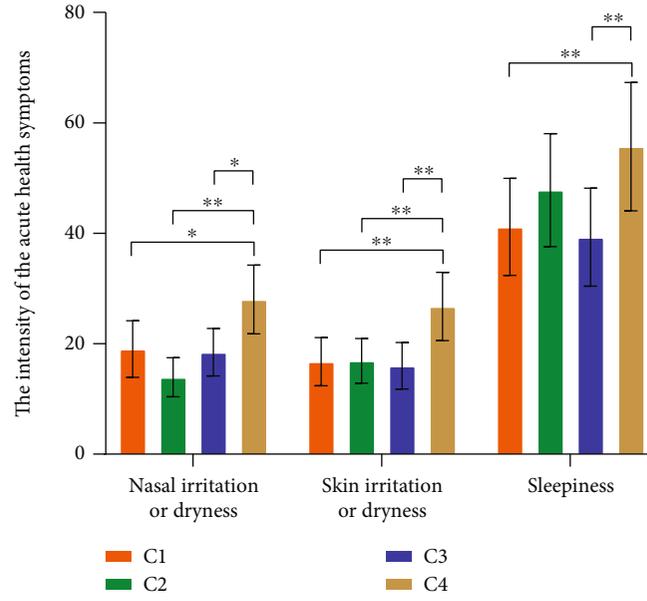


FIGURE 5: Estimated marginal means of acute health symptoms intensity; the bars show the 95% confidence interval (CI) (C1: NAIs+500 ppm CO₂, C2: 500 ppm CO₂, C3: NAIs+2500 ppm CO₂, and C4: 2500 ppm CO₂).

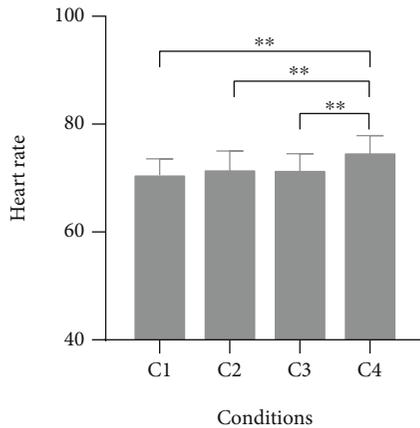


FIGURE 6: Estimated marginal means and the 95% CI of heart rate under the four conditions (C1: NAIs+500 ppm CO₂, C2: 500 ppm CO₂, C3: NAIs+2500 ppm CO₂, and C4: 2500 ppm CO₂).

CO₂ exposure, but only the Rotation score in reasoning skill showed a significant decrease. The results of previous studies on the effect of CO₂ on cognitive performance were inconsistent, and about half of the studies found significant changes in cognitive test scores when pure CO₂ was injected into the test environments [41]. The addition of pure CO₂ may influence aspects of cognitive performance after only short exposures [17]. A drop of over 10% in at least 7 out of 9 Strategic Management Simulation battery scores was observed in studies using the SMS battery at pure CO₂ concentrations as low as 1000 [42] or 1400 [42] ppm. Sayers et al. [43] found that reasoning skills were poorer after adding pure CO₂. Our results support that pure CO₂ affects human cognitive performance. Our results showed that exposure to NAIs+500 ppm CO₂ leads to better cognitive performance in reasoning (Odd One Out), short-term mem-

ory (Spatial Span), and verbal (Digit Span) skills compared to exposure to 500 ppm CO₂. These findings are consistent with those of previous studies. NAIs have many benefits in brain function [44]. Research on the effects of NAIs on cognitive function in young male adults found that participants in the NAI session demonstrated shorter reaction times and higher accuracy for both Stroop congruent and incongruent trials [23]. However, the biological mechanism by which NAIs lead to improvements in cognitive performance has not yet been clarified.

This study found that the benefits of NAIs on cognitive performance are even more notable under high CO₂ exposure. Exposure to NAIs+2500 ppm CO₂ resulted in increased cognitive performance in reasoning, short-term memory, and verbal skills. All cognitive performance scores of college students under the NAIs+500 ppm CO₂ condition were similar to those under the NAIs+2500 ppm CO₂ condition. This is likely because inhalation of more CO₂ leads to CO₂ retention in the blood and increases the bicarbonate content in the blood. When CO₂-rich blood reaches the brain, changes in pH and bicarbonate content are detected [45], leading to higher arousal in the brain, which can lead to a reduction in cognitive performance [18]. Adjustment to arousal may account for the more positive effect of NAIs under increased CO₂ exposure [23].

This study tested the cognitive performance of college students after 1 and 3 h of exposure to different conditions. The results showed that the cognitive performances after 3 h of exposure were higher than those measured after 1 h of exposure to 500 ppm CO₂ and 2500 ppm CO₂ conditions. This result is reasonable. Cognitive performance in the second test should be better than that in the first test because of the learning effect. However, the cognitive performances after 3 h of exposure were lower than those after 1 h of exposure in the NAIs+500 ppm CO₂ and NAIs+2500 ppm CO₂

conditions. Although the difference in scores between the two sessions was not significant, the expected learning effect did not exist in these two conditions. This may be because, with the increase in exposure time, the improvement of NAIs on cognitive performance is reduced.

Our results showed that exposure to 2500 ppm CO₂ resulted in increased symptoms of nasal irritation or dryness and skin irritation or dryness compared with exposure to 500 ppm CO₂. This result is consistent with that of previous studies. A questionnaire survey in bedrooms showed that higher CO₂ concentrations were significantly associated with a higher percentage of perceived stuffy odors and skin symptoms [46]. Stronger respiratory irritation symptoms after continuous exposure to 3297 ppm CO₂ for 3 h have been reported [47]. The addition of NAIs to a 500 ppm CO₂ environment did not have positive health effects. Compared to 500 ppm CO₂ exposure, NAIs+500 ppm CO₂ exposure resulted in a slight aggravation of nasal irritation or dryness. A previous study also showed that NAI generators in a “sick building” do not improve sick building syndrome symptoms [48]. Interestingly, however, adding NAIs under 2500 ppm CO₂ significantly improved nasal irritation or dryness, skin irritation or dryness, and sleepiness. The benefits of NAIs on acute health symptoms were observed only in the 2500 ppm CO₂ background.

Our study also found that exposure to 2500 ppm of CO₂ increased heart rate. Similarly, NAIs could counteract the increased heart rate caused by exposure to 2500 ppm CO₂. However, positive health effects of adding NAIs to 500 ppm of CO₂ were not observed. Previous studies on the effects of negative ions on heart rate changes have not reached a unified conclusion. Liu et al. [49] found that, among children, an increase in NAIs deteriorated heart rate variability. NAI exposure also had no consistent effect on the heart rate in rats [50].

Our study has some limitations. First, the indoor CO₂ concentration was manipulated by supplying CO₂ through a ventilation outlet, which may fail to achieve a uniform distribution of the indoor CO₂ concentration. Second, NAIs charge PM by producing a strong electric field that causes the movement of charged particles toward interior surfaces [51]. Therefore, the concentration of PM is very low in the high NAI condition. Elevated PM pollution levels significantly affect short-term cognition [52]. The improvement of cognitive function in high NAI conditions may be partly caused by the decrease in PM concentration. However, since the decrease in PM concentration is the inevitable result of adding NAI indoors, we can still be sure that the improvement of cognitive performance and health in this study is caused by the increase of NAIs indoors. Finally, due to the different scoring criteria and difficulty of different cognitive tests, the scores of 12 cognitive tests vary greatly and are not easy to compare the difference in the cognitive level under different CO₂ and NAI conditions. Therefore, we standardized the scores using the average score of the same person on a particular cognitive test. However, this standardized treatment cannot reflect multiple testing across outcomes and timing. This study demonstrated that the addition of NAIs in classrooms with high CO₂ concentrations significantly enhanced college students’ cognitive per-

formance and improved acute health symptoms, and this benefit was greater than the effect of adding NAIs to a normal CO₂ condition.

5. Conclusion

This study investigated the effects of adding NAIs to a high pure CO₂ environment on the cognitive performance and health symptoms of college students. Our main conclusions are as follows:

- (i) Adding NAIs in a 500 ppm CO₂ condition resulted in better performance for reasoning and verbal skills, but there were no observed health benefits
- (ii) Compared with exposure to 500 ppm CO₂, exposure to 2500 ppm CO₂ leads to a worse reasoning skill performance, more serious self-reported nasal irritation or dryness and skin irritation or dryness, and increased heart rate
- (iii) The addition of NAIs at 2500 ppm CO₂ improved reasoning, short-term memory, and verbal skills of college students and improved nasal irritation or dryness, skin irritation or dryness, sleepiness, and heart rate to the same level as exposure to NAIs+500 ppm
- (iv) The benefits of adding NAIs in high CO₂ condition on cognitive performance are more noticeable than those in low CO₂ condition. The positive effects of NAIs on cognitive performance may decrease over time

Therefore, adding NAIs to a high pure CO₂ concentration can raise the cognitive performance of college students higher than that in a normal environment and reduce the symptoms of nasal irritation or dryness, skin irritation or dryness, sleepiness, and heart rate.

Data Availability

Data are available on request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Supplementary Materials

The supplementary material contains three figures describing the cognitive performance scores, acute health symptom intensity scores, and health indicator levels that did not change significantly under four environmental conditions. These results are used as a supplement to the paper. (*Supplementary Materials*)

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