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Effects of exercise and poor indoor air quality on learning, memory and blood IGF-1 in adolescent mice

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Abstract

It is known that regular aerobic exercise enhances cognitive functions and increases blood insulin-like growth factor 1 (IGF-1) levels. People living in urban areas spend most of their time indoors and indoor air quality can affect health. We investigated the effects of aerobic exercise in poor and good air quality environments on hippocampus and prefrontal cortex (PFC) neurons, anxiety, and spatial learning and memory in adolescent mice. Poor air quality impaired spatial learning and memory; exercise did not affect learning or memory impairment. Exercise in a good air quality environment improved spatial learning and memory. Poor air quality increased apoptosis in the hippocampus and PFC. Both exercised and sedentary groups living in a poor air quality environment had lower serum IGF-1 levels than those living in a good air quality environment. Living in a poor air quality environment has negative effects on the hippocampus, PFC and blood IGF-1 levels in adolescent mice, but exercise did not alter the negative effects of poor air quality.

Key words: adolescent, air quality, carbon dioxide, exercise, hippocampus, IGF-1, learning, memory, prefrontal cortex

It is known that regular aerobic exercise improves brain function by enhancing cerebrovascular integrity and increasing capillary growth and dendritic connections (Dodge and Rahamimoff 1967). Aerobic exercise in young and adult rats increases their cognitive functions and blood insulin-like growth factor 1 (IGF-1) levels (Radak et al. 2001, Uysal et al. 2005a).

In industrialized countries, more than 85% of the population live in cities. People in cities spend approximately 90% of their time indoors (Soysal and Demiral 2007). Outdoor sport facilities are scarce owing to urbanization; therefore, people tend to exercise in indoor sports facilities, which often do not control air quality.

Indoor air quality (IAQ) refers to the condition of air within buildings. To qualify as "good" IAQ, the temperature should be 19–23° C, relative humidity 40–60% and airflow rate 0.1 m/sec (American Society of Heating, Refrigerating and Air Conditioning Engineers (ASHRAE) 1999, Stellman 1998). There are two main methods for assessing IAQ: measuring carbon dioxide (CO_2) levels and measuring the gas mixture (ASHRAE 1999).

Poor IAQ may affect health adversely. Increased environmental concentration of CO_2

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causes a condition known as "sick building syndrome." Sick building syndrome is characterized by subjective symptoms including irritation of the eyes, nose and throat; headache; and general fatigue (ASHRAE 1999, National Institute for Occupational Safety and Health (NIOSH) 1998, Stellman 1998).

Humans are the main source of indoor carbon dioxide. The ASHRAE and NIOSH recommend that carbon dioxide levels not exceed 700 ppm (ASHRAE 1999, NIOSH 1998). The acceptable limit of ambient CO_2 concentration is 1000 ppm. CO_2 levels should not exceed 1500 ppm according to United Kingdom standards and 3500 ppm according to European standards. The Occupational Safety and Health Administration (OSHA) limits CO_2 concentration to 5000 ppm in the workplace (NIOSH 1998).

Children are more susceptible than adults to the adverse effects of indoor air pollution. Eighty percent of alveoli develop postnatally and development of the lungs continues until 6–8 years of age (Dietert et al. 2000, Plopper and Fonucchi 2000). Brain development begins *in utero* and continues until the end of the adolescent period (Rice and Barone 2000). Developing cells are affected by both internal and external factors (Uysal et al. 2005a,b, 2012, Tugyan et al. 2006).

We investigated the effects of aerobic exercise in good or poor air quality environments on hippocampus and prefrontal cortex (PFC) neurons, the level of anxiety and spatial learning, and memory and blood IGF-1 levels in adolescent mice.

Materials and methods

Animals

Twenty-two-day-old Balb/c mice were purchased from Experimental Animal Laboratory of Dokuz Eylul University (Izmir, Turkey). The animals were subjected to 500 or 3000 ppm CO_2. Three thousand ppm is the ambient level of CO_2, which is below the threshold for defining poor air quality according to the European standard; 500 ppm is below the 650 ppm that is considered clean air by ASHRAE. The mice were separated into four groups of seven animals: (1) exercised while exposed to 500 ppm CO_2, (2) sedentary while exposed to 500 ppm CO_2, (3) exercised while exposed to 3000 ppm CO_2, and (4) sedentary while exposed to 3000 ppm CO_2. Each group included both female and male mice. The animals were maintained with a 12 h light/dark cycle, and food and water were available *ad libitum* during the seven week experiment. All experiments were performed in accordance with the guidelines and approval of the Animal Care and Use Committee of the Dokuz Eylul University School of Medicine.

Air quality apparatus

All animal cages were placed in an apparatus that controlled the input and output of air, and maintained CO_2 at the desired level (Fig. 1). The exhaled air of the animals was the source of carbon dioxide in this apparatus.

Exercise protocol

Mice in the exercise groups were given free access to a running wheel (diameter 11.5 cm) connected to a digital counter; their exercise was voluntary for 6 weeks.

Open field test

Anxiety levels of the animals were evaluated using the open field test. The open field test apparatus was 1×1 m and 50 cm high; a video camera was installed 2.5 m above the apparatus. Each mouse was placed in the center of the open field, then ambulation was recorded in a sound-proof observation room illuminated with light (100 lux) controlled by a light meter (RP electronics, Vancouver, Canada). The analysis arena was divided into 16 squares consisting of 12 peripheral squares and four central squares. The distance traveled by the animal along the walls, sides and center of the arena was recorded over a period of 5 min. The time spent in the center part of the apparatus was calculated also, because rodents characteristically prefer the periphery of the apparatus to the central parts of the open field. Total exploratory time was measured by total number of squares crossed. Increased total exploratory time (increased percentage of squares used) and increased time spent in the central part were considered an index of anxiolysis (Casarrubea et al. 2013, Aksu et al. 2012, Baykara et al. 2013).

Morris water maze test (MWM)

After the open field test, all mice were subjected to the MWM test. The MWM was 140 cm in diameter and 75 cm deep. The water level in the tank was 50 cm, which was 1.0 cm above the height of an invisible escape platform. Each mouse was

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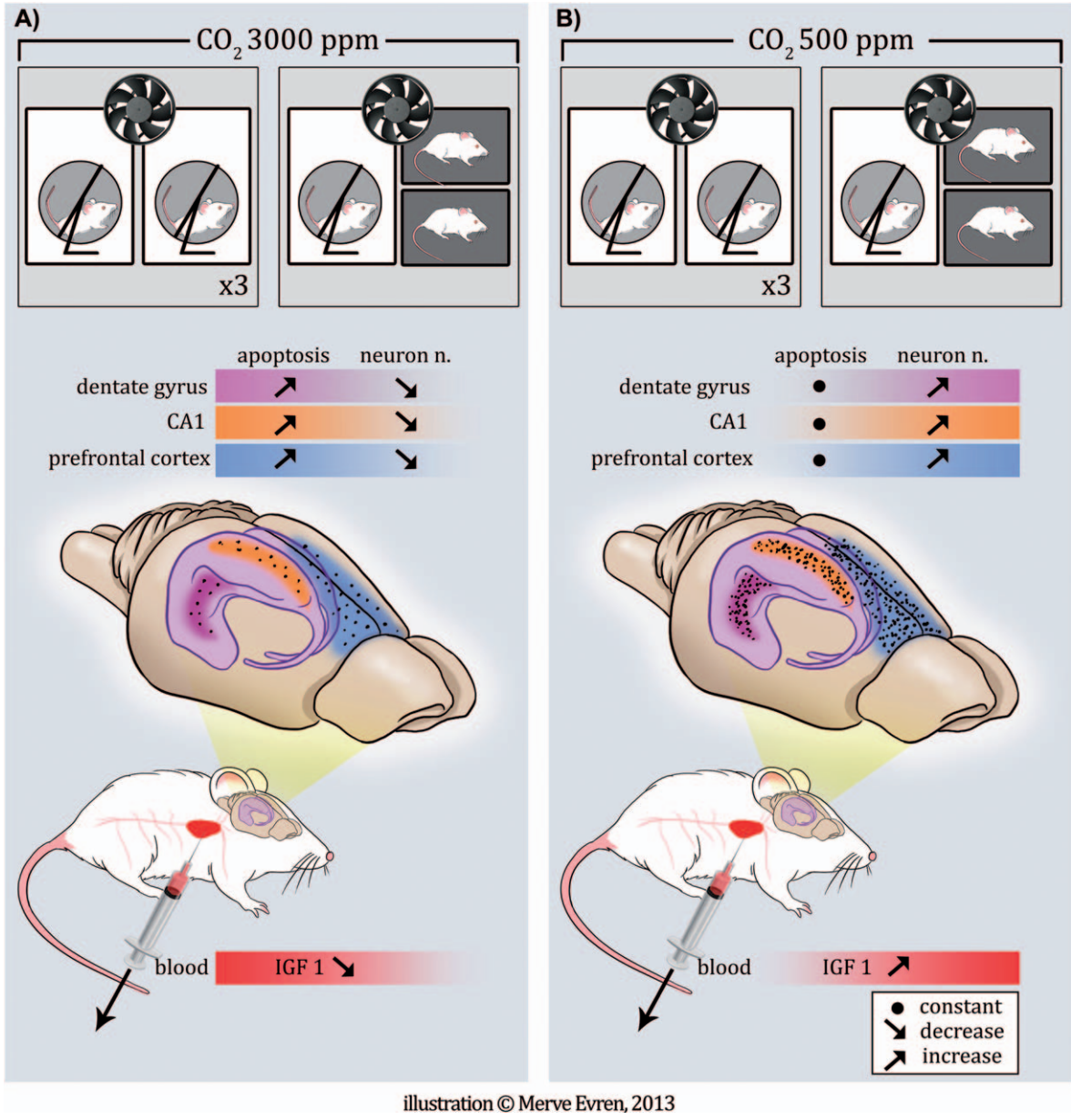


Fig. 1. Schematic diagram of experiment.

exposed to the task for four consecutive days (total 20 trials), and on day five, a probe trial was run. In this task, each mouse was placed in the water and expected to locate the platform within 60 sec. During the probe trial, the platform was removed and the total times spent in the quadrant where the platform originally was located and in the quadrant opposite the original platform location were recorded. A different initiating point (north, south, east, west) was chosen for different days of the experiment. Platform location time, swimming speed and total swimming distance during a 4 day period were recorded for each animal. MWM and open field tests were conducted in a fresh air environment and recorded using a HVS video-tracking-system (Buckingham, UK).

Biochemical analysis

After the MWM test, the mice were sacrificed after light ether anesthesia, blood was drawn, and the brains were removed. IGF-1 protein levels in the serum were measured using a commercially available ELISA kit specific for rat IGF-1 according to the manufacturer's instructions (Catalog no. EK0377; Boster Immunoleader,

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Wuhan, China) with assay sensitivity <5 pg/ml and a range of 62.5–4000 pg/ml).

Microscopic examination

The brain was sectioned coronally into sequential 5 μm sections using a microtome (Leica RM2245, Wetzlar, Germany). Neuron density was measured for each mouse using three coronal sections through the hippocampus that corresponded to plates 21, 23, 25 and through the PFC that corresponded to plates 9 and 11 of the rat atlas of Paxinos and Watson (1982). All sections were stained by cresyl violet. The images were analyzed using a computer assisted image analyzer system consisting of a microscope (Olympus CX41, Tokyo, Japan) equipped with a high resolution video camera (Olympus DP71). The density of hippocampal neurons was determined using a 16800 μm^2 counting frame viewed through a $40\times$ Nikon lens on the monitor. The PFC and hippocampal neurons were counted in CA1, CA2, CA3 and gyrus dentatus (GD) regions and neuron density was calculated.

The DeadEnd Colorimetric TUNEL system kit (Roche, Mannheim, Germany) was used to detect apoptotic cells. TUNEL staining was performed using an In Situ Cell Death Detection Kit® (Roche) according to the manufacturer's protocol. Briefly, sections were deparaffinized, hydrated through a series of alcohols, washed in distilled water and phosphate-buffered saline (PBS), and de-proteinized using proteinase K (20 μg/ml) for 15 min at 37° C. The sections were rinsed, then incubated in the TUNEL reaction mixture. The sections were rinsed, then visualized using converter-POD with 0.02% 3,3'-diaminobenzidine (DAB). The sections were counterstained with hematoxylin. Five fields were chosen randomly for each slide and the TUNEL positive cells per field were counted. The apoptotic index (percentage of apoptotic nuclei) was calculated as apoptotic nuclei/total nuclei counted $\times 100$. All counting procedures were performed blindly.

Statistical analysis

Differences in the behavioral and biochemical parameters among the groups were analyzed using two-way ANOVA and post hoc comparisons were done using Bonferroni. Differences between the learning days for the MWM were analyzed using GLM repeated measure. Correlations among groups were calculated using Pearson correlation analysis. Results are presented as means $\pm$ SEM and differences were considered significant at $p \leq 0.05$.

Table 1. Exercise duration and distance

CO_2 level	Running time (min)	Running distance (km)
500 ppm + exercise	163 ± 1.08	2.7 ± 0.1
3000 ppm + exercise	79 ± 1.01*	1.4 ± 0.08*

*$p < 0.001$ compared with 500 ppm + exercise.

Results

Animal exercise

Mice that lived in a good air quality environment (500 ppm CO_2) exercised for longer times and longer distances than those that lived in a poor air quality environment (3000 ppm CO_2) ($p < 0.001$; Table 1).

Open field test

In the open field test, the percentage of squares used by the sedentary group in the 3000 ppm CO_2 environment was lower than the other groups ($p < 0.05$) (Fig. 2A). Both sedentary and exercise groups that lived in the 3000 ppm CO_2 environment ambulated less in the center of the open field compared to both sedentary and exercise groups that lived in the 500 ppm CO_2 environment ($p < 0.001$ for both groups) (Fig. 2B).

MWM test

The MWM test showed that voluntary exercise in a 3000 ppm CO_2 environment significantly impaired spatial learning and memory. The mean latency to find the platform decreased progressively in animals that lived in a 500 ppm CO_2 environment ($p < 0.05$); however, this reduction was not observed in groups that lived in a 3000 ppm CO_2 environment (both sedentary and exercise groups) ($p > 0.05$). Mice that lived in the 3000 ppm CO_2 environment (sedentary and exercise) had longer escape latencies on the third and fourth days of training days than those that lived in a 500 ppm CO_2 environment (third day, $p < 0.05$; fourth day, $p < 0.005$) (Fig. 3A).

In probe trials, time spent in the target quadrant was used to evaluate long-term memory. Animals that lived in a 500 ppm CO_2 environment (both sedentary and exercise) spent more time in the target quadrant than in the opposite quadrant compared to those that lived in a 3000 ppm CO_2 environment (target quadrant, $p < 0.001$; opposite quadrant, $p < 0.05$) (Fig. 3B). Mice in the exercise group that lived in a 500 ppm CO_2 environment spent more time in the

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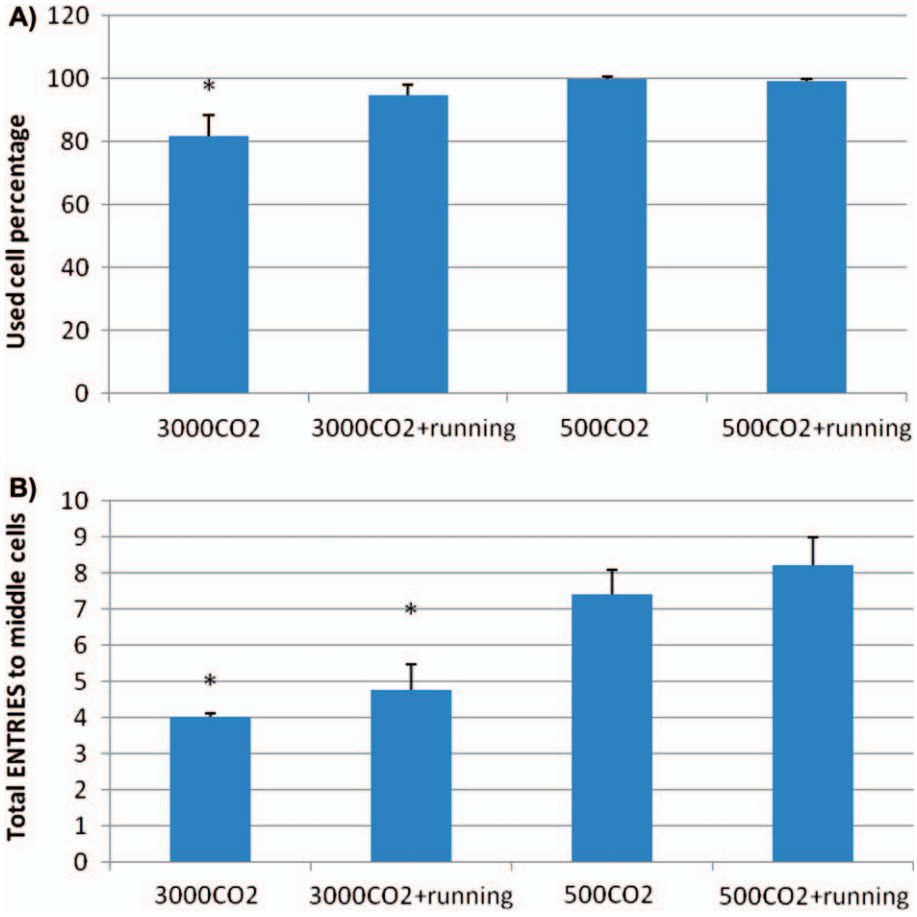


Fig. 2. Open field test results. A) Percentage of cells used in the open field arena; *$p < 0.05$ compared with all other groups. B) Number of entries into the middle cells; *$p < 0.05$ compared to the 500 ppm CO_2 and the 500 ppm CO_2 + exercise groups.

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target quadrant compared to sedentary mice that lived in a 500 ppm CO_2 environment ($p < 0.002$). There was no significant difference between exercise and sedentary groups that lived in a 3000 ppm CO_2 environment regarding the time spent in the target and opposite quadrants ($p > 0.05$).

Histological and biochemical tests

The densities of neurons in the PFC and GD were significantly reduced in mice that lived in a 3000 ppm CO_2 environment compared to those that lived in a 500 ppm CO_2 environment (PFC, $p < 0.001$; GD, $p < 0.004$) (Fig. 4A, B). In addition, GD and PFC neuron numbers increased in the 500 ppm + running group compared to the 500 ppm group (GD, $p < 0.002$; PFC, $p < 0.004$) (Fig. 4A, B).

TUNEL positive neurons were significantly more numerous in mice that lived in a 3000 ppm CO_2 environment (both sedentary and exercise) than in those that lived in 500 ppm CO_2 environment (both sedentary and exercise) in both the PFC and hippocampus regions (all regions, $p < 0.0001$) (Fig. 4C, D; Table 2). Both exercise and sedentary groups that lived in a 3000 ppm CO_2 environment had lower serum IGF-1 levels than those that lived in a 500 ppm CO_2 environment (both sedentary and exercise) (Table 3). We found no statistically significant differences between body weights and corticosterone levels among groups (Table 3). We also observed a strongly negative correlation between blood IGF-1 levels and TUNEL positive neuron percentages (Fig. 4E; Table 4).

Discussion

Ours appears to be the first study of the effects of poor IAQ on the brain. We found that living in a 3000 ppm CO_2 environment impaired learning and memory performance, increased apoptosis in PFC and hippocampal neurons and decreased

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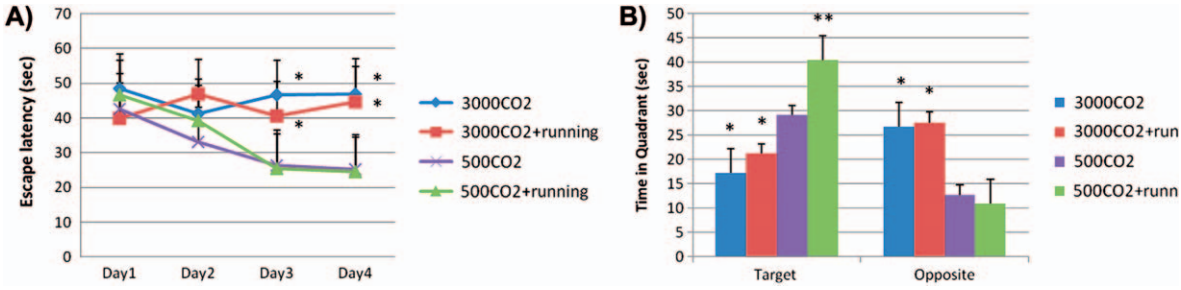


Fig. 3. The MWM performance of the groups. A) Daily change in escape latency. B) Time spent in target and opposite quadrant in probe trial; *$p < 0.05$ compared to 500 ppm CO_2 and 500 ppm CO_2 + exercise groups. **$p < 0.05$ compared to all other groups.

serum IGF-1 levels in adolescent mice; exercise did not alter these negative effects. The criterion for learning in the probe trial is that a total of 36% of the time should be present in original platform location (Morris 1984). Our 3000 ppm groups, both sedentary and exercised, spent less than 36% of their time in the target quadrant (original platform location) and more time in the opposite quadrant (opposite the original platform location) in a probe trial, which means they did not learn the location of platform. We found only one study that related air pollution to behavior. In that study, living near a roadway for 10 months was linked to impaired spatial learning and depression (Fonken et al. 2011).

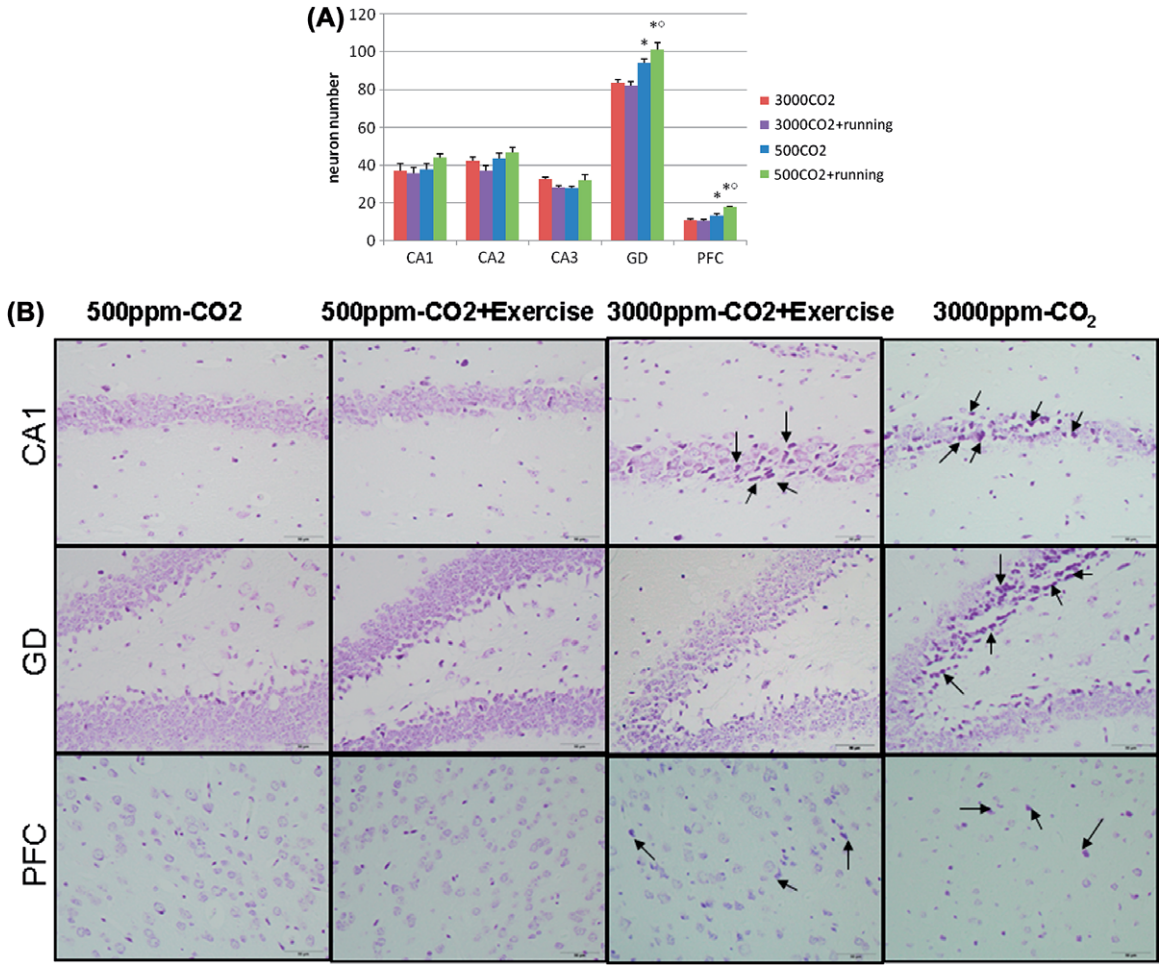


Fig. 4. Neuron numbers and apoptosis of hippocampus and PFC. A) Total number of neurons. B) Representative photomicrographs of neurons of hippocampus and PFC.

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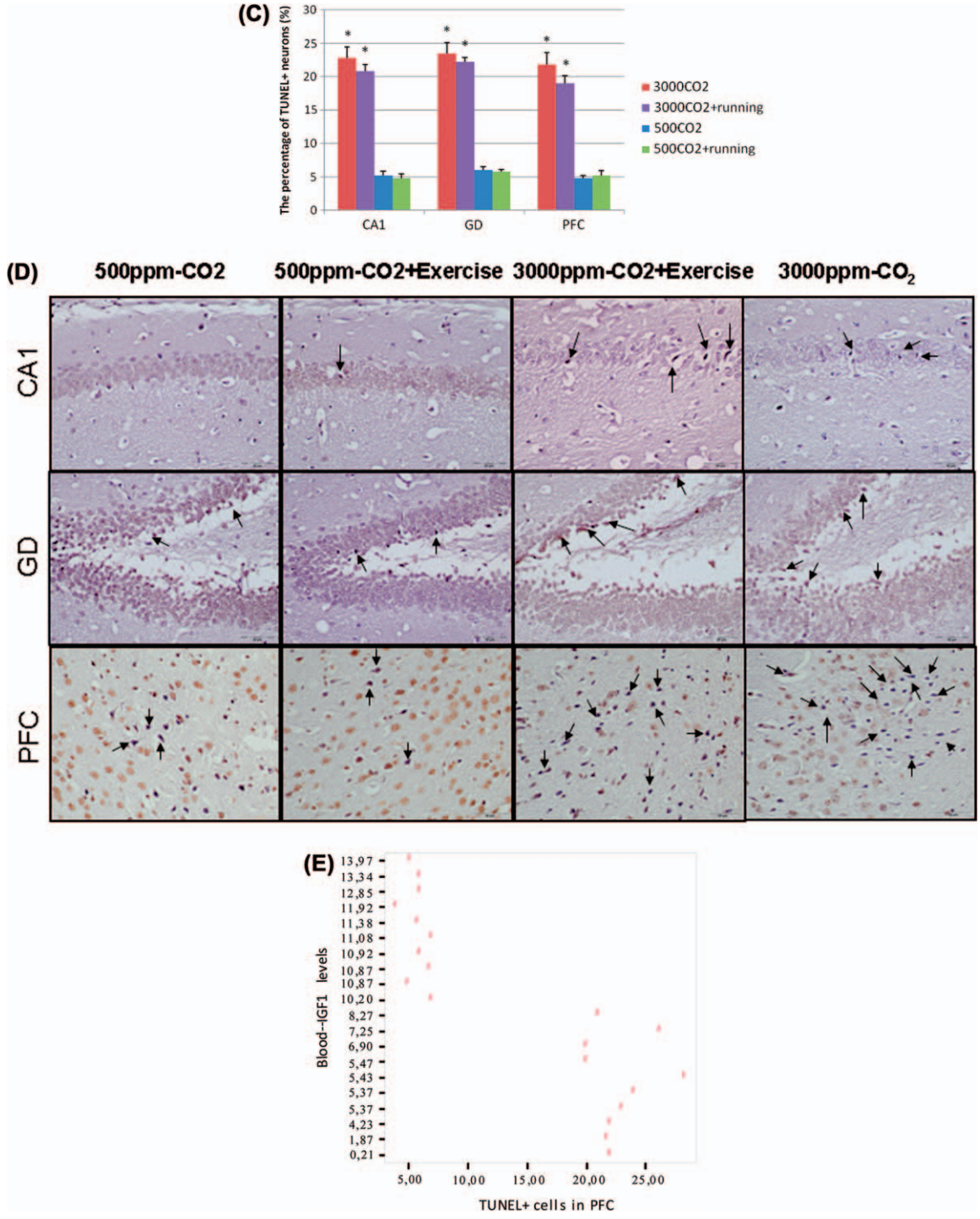


Fig. 4 (Continued). C) The percentage of TUNEL positive neurons. D) Representative photomicrographs of TUNEL stained sections. E) Correlation between blood IGF-1 levels and TUNEL positive neuron percentage in prefrontal cortex; $^{*}p < 0.05$ compared to 500 ppm CO_2 group. GD, gyrus dentatus; PFC, prefrontal cortex.

All organs produce IGF-1; the liver produces approximately 70% of the total circulating IGF-1 (Torres-Aleman 2010). IGF-1 crosses the blood-brain barrier; therefore, the brain is influenced by the concentration of circulating IGF-1 (Carro et al. 2000). IGF-1 plays a major role in brain development (Aleman and Torres-Alemán 2009). Circulating IGF-1 influences hippocampal IGF-1 levels and brain gene expression in adolescent rats (Yan et al. 2011). Reduced circulating IGF-1 has been

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Table 2. TUNEL positive cell numbers of hippocampus and prefrontal cortex of mice.

	TUNEL positive cells (%) (mean ± SEM)		
CO_2 level	CA1	GD	PFC
500 ppm	5.2 ± 0.66	5.76 ± 0.31	4.8 ± 0.37
500 ppm + exercise	4.8 ± 0.66	6 ± 0.54	5.2 ± 0.73
3000 ppm	22.8 ± 1.65*	23.46 ± 1.60*	21.8 ± 1.77*
3000 ppm + exercise	20.8 ± 0.96*	22.2 ± 0.66*	19 ± 1.14*

*$p < 0.05$ compared to 500 ppm and 500 ppm + exercise. GD, gyrus dentatus; PFC, prefrontal cortex.

implicated in the development of cognitive dysfunction in adults and adolescents (Aleman and Torres-Alemán 2009, Ozdemir et al. 2012, Baykara et al. 2013, Aksu et al. 2012). We found that living in a 3000 ppm CO_2 environment reduced blood IGF-1 levels.

IGF-1 may play a role in amelioration of memory deficits. It is known that circulating IGF-1 influences tissue IGF-1 levels in the brain and causes behavioral alterations (Trejo et al. 2007). An important question is whether circulating IGF-1 levels are predictive of cognitive dysfunction that results from living in a 3000 ppm CO_2 environment in developing brain. In animal models, it has been shown that decreased serum IGF-1 levels were associated with cognitive deficits (Trejo et al. 2007, Aksu et al. 2012, Baykara et al. 2013) and that IGF-1 deficiency led to impaired learning and memory in adulthood (Sonntag et al. 2005). We found that mice that lived in a 3000 ppm CO_2 environment had lower levels of IGF-1 and impaired learning and memory compared to those living in a 500 ppm CO_2 environment.

IGF-1 prevents apoptosis and serum IGF-1 levels are related negatively to tissue damage (Wine et al. 2009, Aksu et al. 2012, 2013). We found a strongly negative correlation between blood IGF-1 levels and TUNEL positive neurons. Apoptosis eliminates cells (Kerr et al. 1972). Apoptosis increases in brains of

Table 4. Correlation between blood IGF-1 and TUNEL positive neurons in hippocampus and PFC

	Blood IGF-1
TUNEL+ cell CA1	$r = -0.836$, $p < 0.0001$
TUNEL+ cell GD	$r = -0.854$, $p < 0.0001$
TUNEL+ cell PFC	$r = -0.840$, $p < 0.0001$

mouse pups when exposed to 8% CO_2 between postnatal days 2 and 7 (Das et al. 2009). We observed that living in a 3000 ppm CO_2 environment for a long period increased apoptosis in all regions of hippocampus and PFC.

The number of cells in the brain of adolescents is a result of the equilibrium of cell proliferation and apoptosis. External factors can affect both cell proliferation and death (Uysal et al. 2005a,b, 2012, Tugyan et al. 2006). We showed that living in a 3000 ppm CO_2 environment caused significant reduction in the number of neurons in the GD and the PFC, and increased apoptosis in the CA1 and GD regions of the hippocampus and the PFC. These results indicate that the cortex may be affected more adversely than other regions of brain by poor IAQ. Also, it is known that apoptosis is increased by hypoxia/hypercapnia in the cerebral cortex (Douglas et al. 2010).

CO_2 concentration exerts an important influence on intra- and extracellular pH. CO_2 passes quickly through the cell membranes to form carbonic acid with H_2O, which releases H^+ ions and, in excess, causes acidosis (Sikter 2007). Acidosis decreases transmembrane Ca^{+2} conductance and decreases the excitability of neurons (Dodge and Rahamimoff 1967, Tombaugh and Somjen 1997). Calcium overload causes excitotoxicity and apoptosis during hypoxia (Hota et al. 2011). When exposure to CO_2 is prolonged (several hours to one week), the organism depletes its buffer systems. The pH balance of cells and the extracellular space are maintained by the kidneys and tissue buffer mechanisms. (Sikter et al. 2009). The development of a new ionic milieu requires 5–7 days to restore the equilibrium of the cells (Gennari et al. 1972). Chronic hypercapnia

Table 3. Body weight, blood IGF-1 levels and corticosterone levels

CO_2 level	Body weight (g)	Blood corticosteroid levels (pg/ml)	Blood IGF-1 levels (pg/mg protein)
500 ppm	18.8 ± 1	0.08 ± 0.08	11.6 ± 0.6
500 ppm + exercise	17.7 ± 0.8	0.10 ± 0.04	11.9 ± 0.6
3000 ppm	19.5 ± 1	0.16 ± 0.05	4.1 ± 1*
3000 ppm + exercise	18.2 ± 0.8	0.18 ± 0.04	5.9 ± 1.1*

*$p < 0.05$ compared to 500 ppm and 500 ppm + exercise.

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is followed by cascades that alter the ionic milieu in the cells and may alter the neurotransmitter/endocrine status (Dodge and Rahamimoff 1967).

We observed that the mice in the 3000 ppm CO_2 groups (both sedentary and exercise) were less active in the open field test and ambulated less in the center of the open field area compared to mice in 500 ppm CO_2. Mice, by their nature, exhibit the exploratory behavior. It is widely accepted that in a square open field arena, time spent on the corners is correlated with rodent anxiety. Our finding suggests that the anxiety of mice living in the 3000 ppm CO_2 environment was greater than those living in the 500 ppm CO_2 environment.

If the CO_2 level in a room is low, the air quality is considered good (ASHRAE 1999). Living organisms are the most important source of carbon dioxide in the environment. In fitness centers, equipment often is placed in relatively small spaces and many people use the space at the same time. Hyperventilation during exercise quickly causes ambient air pollution. Our model was selected to parallel air conditions in crowded fitness centers.

The motivation among humans for voluntary exercise is related to personal characteristics and rewards (psychological or physical) (Rhodes and Smith 2006, Brene et al. 2007). In rodents, motivation is derived from community effects. The running wheel is a reward for rodents (Jonas et al. 2010) and can be addictive (Brene et al. 2007, Kanarek et al. 2009). It is known that dopamine and serotonin are related to motivation, reward and control of motor behavior (Meeusen et al. 2006). Central dopamine receptor blockade with alteration of dopamine transmission impairs the running performance of rats (Balthazar et al. 2010). Central noradrenergic neurons modulate humor and motivation, and when their activity is low, motivation decreases. This section is difficult to relate to your study, because you studied none of the factors described. Unfortunately, there is no information concerning chronic CO_2 exposure and motivation. We found that the mice living in a 3000 ppm CO_2 environment exercised less than those in the 500 ppm CO_2 group.

Ours is the first study of the effects of high and low levels of CO_2. Living in a 3000 ppm CO_2 environment reduced blood IGF-1 levels and increased apoptosis in the hippocampus and PFC, which resulted in impaired spatial learning and memory. Exercise did not change these negative effects, however. In addition, living in a 3000 ppm CO_2 environment decreased the amount of voluntary physical exercise. Future studies should explore the cause of decreased duration of exercise and the role of the neurotransmitters under conditions of high CO_2 concentration and investigate the effects of different CO_2 levels. Because the developing brain especially may be affected by poor IAQ, further studies should explore the effects of poor IAQ on immature brains.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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