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Effects of Cigarette Smoke Inhalation on Spatial Memory in Mice: The Interplay between Carbon Monoxide and Nicotine

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Abstract

Cigarette smoking is a prevalent recreational activity worldwide, spanning all age groups. While the carcinogenic and addictive aspects of smoking are well-recognized, the neurotoxic effects of other toxins like carbon monoxide (CO) in cigarette smoke are often neglected. CO is a known neurotoxin and is produced in significant quantities during smoking. This study aimed to evaluate the impact of CO gas from cigarette smoke on spatial memory in mice. Sixteen adult male mice were divided into two groups: a control group and an experimental group exposed to cigarette smoke (Aspen brand) in a gas chamber (75x50x50 cm) for 15 minutes daily over 14 days. Each group consisted of 8 mice. CO levels were measured using a digital CO meter (PCMM05 Pyle), and spatial memory was assessed using the Barnes maze protocol. Exposure to cigarette smoke resulted in CO levels of up to 347 ppm and an increase in COHb by 16%, exceeding the WHO's recommended exposure limit of <100 mg/m³ or 87 ppm for 15 minutes and a COHb level of <2%. While spatial memory impairment was observed, the increase in activity likely due to nicotine, a known CNS stimulant, might have mitigated the impact of CO on spatial memory. The memory impairment due to CO exposure might be counteracted by the stimulatory effects of nicotine found in cigarettes.

Keywords: Carbon monoxide, Carboxyhemoglobin, Cigarette smoke, Neurotoxicity, Spatial memory.

INTRODUCTION

Human survival is intricately linked to the natural environment, which provides essential resources (Chivian and Bernstein, 2008). However, human activities often introduce pollutants that can be harmful to health (WHO, 2022). Urban dwellers are particularly susceptible to various chemical, physical, and biological toxins, with air pollution being a significant concern (WHO, 2016). Inhalation is a primary route of exposure, occurring both indoors and outdoors across different environments such as homes, workplaces, and transportation systems (USEPA, 2022).

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Air quality is crucial for health, with clean air necessary for daily life. Ideal air composition includes 78% nitrogen and 21% oxygen, with minimal pollutants (WHO, 2018). However, air pollution introduces harmful substances like nitrogen oxides, sulfur oxides, carbon monoxide (CO), particulate matter, and lead into the atmosphere. These pollutants arise from activities such as combustion and vary based on factors like population density and industrialization (WHO, 2016). Nigeria faces significant air pollution challenges, with varying CO levels across regions. For instance, Abuja (<50 parts per million) has lower CO levels compared to Kano (>50 ppm), likely due to better urban planning and traffic control (Federal Ministry of Environment, 2013).

CO is a prevalent air pollutant globally, responsible for a significant proportion of fatal poisonings. It is a colorless, odorless, tasteless and non-irritating gas that is poorly soluble in water (H_2O) and slightly lower in density than air (USEPA, 2021). It is a significant public health problem in both rural and urban areas of both developed and the under developed countries. It may be responsible for more than one half of all fatal poisonings that are reported worldwide (Raub *et al.*, 2000). It is ubiquitously found in every geographical region both in- and outdoors (Health Effects Institute, 2019).

Cigarettes, primarily composed of dried tobacco leaves, contain harmful substances like nicotine and CO (U.S. Department of Health and Human Services, 2010). Nicotine is a stimulant affecting the cardiovascular system, while CO is a significant indoor pollutant from smoking (USEPA, 2018). With over 4000 constituents in cigarette smoke, many are carcinogens formed during combustion (Rodgman and Perfetti, 2009; Hecht, 2012). Exposure to environmental tobacco smoke (ETS) is often unavoidable, especially for non-smokers living with smokers, posing significant health risks (U.S. Department of Health and Human Services, 2006).

The mechanisms through which cigarette smoke could affect spatial memory includes but are not limited to oxidative stress (Reuter *et al.,* 2010), inflammation (Heffernan and Neill, 2012; Hagiwara *et al.,* 2021), nicotine's dual role (Mansvelder and McGehee, 2000; Kenney and Gould, 2008), dopamine (Alkam *et al.,* 2007; Tizabi *et al.,* 2013), glutamate and GABA (Lubin and Ren, 2007; Tuesta *et al.,* 2011) neurotransmitter disruptions, hippocampal atrophy and cortical thinning.

Carbon monoxide (CO) exposure from the environment can be categorized into indoor and outdoor sources. These sources are the main contributors to CO toxicity, typically involving direct inhalation in confined spaces (indoors) or open environments (outdoors) (USEPA, 2016). CO exposure can occur in various settings such as homes, cars, roadside areas, workplaces, and marketplaces (WHO, 2021). These exposures are often subclinical, meaning they go unnoticed but occur regularly, particularly affecting women and children (CDC, 2019). Common, overlooked sources include smoke from burning cigarettes, which can lead to chronic, low-level CO accumulation in the body over a lifetime, potentially resulting in serious health issues that are not easily linked to CO poisoning (USEPA, 2022).

Environmental tobacco smoke in offices and homes can significantly increase CO levels, with an 8-hour average concentration rising by 20-40 ppm (AQC, 1991). In some homes, CO concentrations have been found to be as high as 53 ppm (Orina *et al.,* 2024). Acute, high CO concentrations, reaching up to 100 ppm, can be found in kitchens, where women often spend a lot of time (AQG, 2000). The effects of chronic CO poisoning vary widely among individuals and depend on many factors. Children exposed to CO often show symptoms earlier and

recover faster than adults due to their smaller blood volume and higher minute ventilation relative to their body mass (Sunny, 2008).

Exposure to carbon monoxide (CO) from indoor sources like cigarette smoking can be extremely dangerous. Carbon monoxide is able to penetrate every cell membrane in the body and cross barriers such as the blood-brain barrier (BBB), reaching the brain at toxic levels (Raub *et al*., 2000). This is particularly harmful because hypoxia (lack of oxygen) is a severe threat to the brain, especially during its development.

Cigarette smoking is a major global source of indoor CO exposure, affecting people across all ethnic, racial, gender, age, social, and economic groups (WHO, 2019). It is a widespread habit that significantly contributes to indoor CO pollution worldwide. The World Health Organization (WHO) has set guidelines for safe CO exposure limits: as 100 mg/m³ (87 ppm) for 15 minutes, 60 mg/m³ (52 ppm) for 30 minutes, 30 mg/m³ (26 ppm) for 1 hour, and 10 mg/m³ (9 ppm) for 8 hours (EPA, 1991).

Carbon monoxide (CO) poisoning leads to 50,000 emergency visits annually in the U.S. and is the leading cause of poisoning deaths there (Hampson *et al*., 1996). In Ibadan, Nigeria, CO levels in some individuals were comparable to those of chronic smokers, with up to 6.5% COHb (Sunny *et al*., 2008). Average indoor CO levels in Kano, Nigeria, ranged from 4.93 to 5.49 µg/m³ (Ayodele *et al*., 2007). Indoor pollution causes nearly 2 million deaths annually in developing countries and contributes 4% to the global disease burden (Bruce *et al*., 2000). In Nigeria, CO poisoning cases are under-reported, with inadequate monitoring and diagnostic tools (Augustine, 2002; Ayodele *et al*., 2007). Chronic CO exposure is linked to dementia, depression, and psychosis (Samuel *et al*., 2007). The U.S. mandates CO alarms in homes and CO monitors near roads (United States EPA, 1991), unlike Nigeria, where such measures are lacking.

This study addresses the gap in understanding chronic CO exposure from cigarette smoke and its effects on spatial memory. Given the widespread use of tobacco and the high levels of CO produced, it's crucial to explore its impact on cognitive functions. The study aims to evaluate the effects of CO exposure from cigarette smoke on spatial memory, contributing to the understanding of its broader health impacts.

MATERIALS AND METHODS

Study Design: Sixteen adult male mice were used in this study. They were housed for one week before the study began to acclimate them to their environment. The mice were kept under natural day and night conditions typical of the savannah region in Kano State, Nigeria, with temperatures ranging from 27°C to 30°C. They were given unrestricted access to laboratory animal feed and water and were handled following Ahmadu Bello University's animal use and care guidelines.

The mice were randomly divided into two groups: the experimental group and the control group, each consisting of eight mice. The experimental group was exposed to cigarette smoke (Aspen brand) in a gas chamber (75 cm x 50 cm x 50 cm) for 15 minutes daily for 14 days. The control group was given similar treatment but without cigarette smoke exposure. This exposure took place each morning between 8-9 am. A digital CO meter (PCMM05, Pyle) measured the CO levels produced by the burning cigarette in the gas chamber, and the peak daily CO levels were recorded. Both environmental and gas chamber temperatures were

documented during each exposure session. The Barnes maze protocol was used to assess the mice's spatial memory.

Measurement of Body Weight: The body weights of all mice were measured and recorded on the first and last days of the study to evaluate weight gain.

Screening for Motor Coordination Deficits: To ensure reliable cognitive assessments, the mice were screened for motor coordination deficits before the study began (Stanley *et al.*, 2005). The balance beam test (Beam Walk) was used to assess motor coordination and balance. This test involved a 100 cm long beam, with widths of 12 mm and 6 mm, elevated 50 cm above a table. A black escape box was attached to one end. Mice were encouraged to move away from an aversive 60-watt light bulb at the start of the beam towards the escape box. During this test, the latency to reach the box and the number of hind-feet slips were recorded as measures of motor coordination and balance (Carter, *et al.*, 2001).

Assessment of Learning and Memory: The Barnes maze consisted of a circular platform (122 cm in diameter) with 40 equally spaced holes (5 cm diameter, 3.5 cm apart, and 2 cm from the edge) around the perimeter, elevated 90 cm above the floor. Under one of the holes was a dark "goal box" (28 x 14 x 18 cm) where mice could escape from the aversive bright light. Visual cues were placed around the room and kept in the same positions throughout the study. A video camera, positioned 150 cm above the platform, recorded the mice's activities (Barnes, 1979).

The study included an adaptation phase followed by four days of training (acquisition/learning), culminating in a probe/retention test on the fifth day. During the acquisition task, the experimenter measured the number of primary errors (head deflections into incorrect holes before reaching the target hole), total errors, primary latency (time to locate the target hole), and path length (total path to locate the target hole) (Harrison and Reiserer, 2009). On the fifth day, they recorded the number of pokes/errors, latency, and path length to reach the virtual target hole (Sunyer, *et al.*, 2007).

Mice search strategies were categorized into three types: direct (spatial) search, mixed search, and serial search (O'Leary, *et al.*, 2011). The direct strategy involved moving straight to or near the target hole before reaching it. The mixed strategy involved crossing through the maze center or an unorganized search. The serial strategy involved visiting at least two adjacent holes in sequence (clockwise or counterclockwise) before reaching the target hole. These strategies were observed by the experimenters and confirmed through video recordings at the end of each day's trials.

Assessment of Carbon Monoxide Level in Blood: About 2.5 ml of blood was collected in test tubes containing ethylenediaminetetraacetic acid (EDTA, potassium salt) at 1.5 mg/mL of blood. Blood carboxyhemoglobin (COHb) levels were measured as the primary biomarker for CO exposure (Beutler and West, 1984).

Statistical Analyses: Data from the study were expressed as means ± standard error of the mean (SEM) or as medians and interquartile ranges. Depending on the nature of the data, either parametric or non-parametric analyses were performed, followed by appropriate post hoc tests if necessary. Statistical significance was set at $p \leq 0.05$. Data analyzed using SPSS software version 23.0.

RESULTS

Effect of Cigarette Smoke Inhalation on Body Weight: Both groups of mice showed normal weight gain throughout the study. This was evidenced by a significant increase in their final body weight compared to their initial body weight for both the cigarette smoke-exposed group (p=0.017) and the control group (p=0.012) (Table 1).

Table 1: Effect of Cigarette Smoke Inhalation on Body Weight

Groups	Initial Body Weight (Mean± SEM)	Final Body Weight (Mean± SEM)	P value
Control	20.9± 0.2	27.5± 0.3	0.012*
Cigarette	21.2± 0.4	27.7± 0.5	0.017*

Wilcoxon Signed Ranks Test, (Z=-2.380, p=0.017). n=8, p≤0.05, * indicates statistical significance, and its absence indicates insignificance.

Temperature Variation During the Study: There was no significant difference (p=0.7) between the temperature inside the gas chamber and the surrounding environment. Thus, both groups were kept under similar temperature conditions throughout the study (Table 2).

Table 2: Temperature variation between the gas chamber and the environment

Location	Gas Chamber	Environment	p-value
Temperature (Mean ^{0}C ± SEM)	27.8 ± 0.4	28.0 ± 0.4	0.7

Independent-Samples T-Test, n=8, p≤0.05, * indicates statistical significance, and its absence indicates insignificance.

Carbon monoxide Exposure and Percentage Carboxyhemoglobin (COHb): The experimental group exposed to cigarette smoke had a mean daily CO exposure of 347 ppm, while the control group had 1.6 ppm. Correspondingly, the COHb levels were 16% in the cigarette group and 2% in the control group. The %COHb levels were directly proportional to the CO exposure (Figure 1).

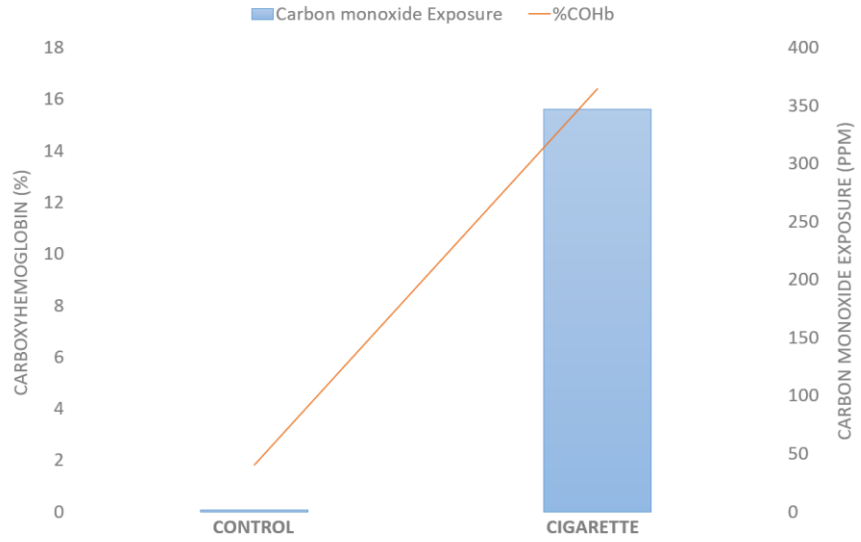

Figure 1: Carbon monoxide exposure in relation to the percentage carboxy-hemoglobin.

Screening for Motor Coordination Deficit: There were no significant differences between the cigarette smoke-exposed group and the control group in terms of latency to finish the beam (p=0.117), left hind-feet slips (LFS, p=0.312), and right hind-feet slips (RFS, p=0.394) (Table 3). This suggests that none of the mice in either group exhibited gross motor coordination or balance deficits, which could have impacted their performance in the Barnes maze tasks.

Table 3: Screening animals for motor coordination deficit

Groups	Control	Cigarette	P value
Left Foot Slip (LFS)	1.50	1.00	0.31
Right Foot Slip (RFS)	0.50	0.81	0.39
Latency (Sec)	57.06	45.25	0.12

Kruskal-Wallis Test indicates no significant difference between all the groups [X^2=3.57 (LFS), 2.99 (RFS), and 5.90 (L)], n=8, $p \leq 0.05$. * Indicates statistical significance, and its absence indicates insignificance.

Assessment of Spatial Memory

Latency: Both the control and cigarette smoke-exposed groups showed significant reductions in primary latencies over time. A significant difference was noted between Day 1 (D1) and Day 5 (D5) for the control group (p=0.001) after post hoc (Figure 2). Although the cigarette group also showed a gradual decrease in latency, their overall latency values remained higher than those of the control group.

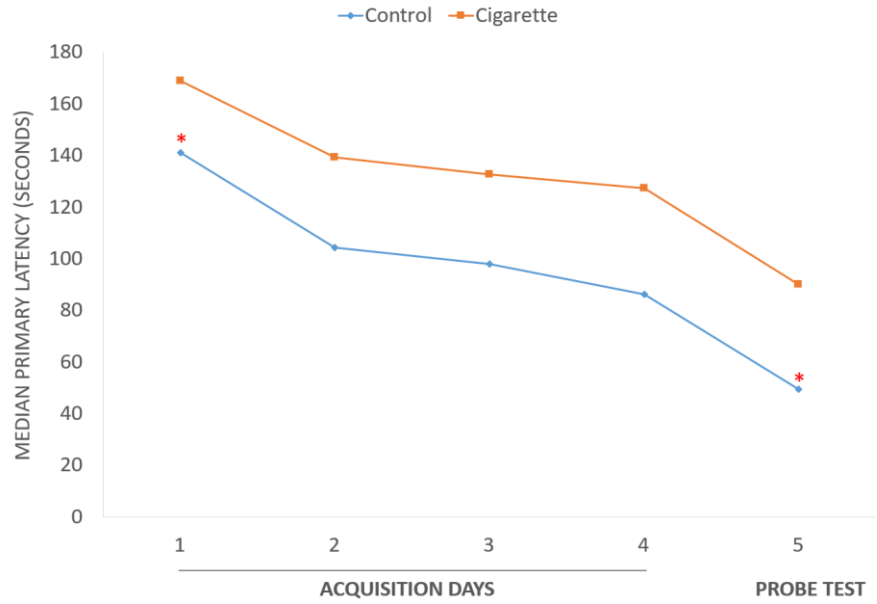

Figure 2: Changes in the primary latency to reach the target hole.

Control: Friedman test indicates significant decrease in the primary latency [χ^2 (4) = 15.895, p = 0.003] that occurred between D3 and D5 (p=0.001) after post hoc comparison. Cigarette: There is a significant change in the latencies [χ^2 (4) = 10.503, p = 0.033], however, no significant difference was observed after post hoc comparison. $p \leq 0.05$, n=8.

Path Length: In the control group, the primary path length (PPL) significantly decreased over the acquisition period (p=0.03) (Figure 3). The cigarette smoke-exposed group exhibited a sharp increase in PPL on Day 2 (D2), followed by a gradual decrease over the subsequent days (Figure 3). The total path length (TPL) significantly decreased in the control group (p=0.013), while it increased in the cigarette group (Figure 3). The cigarette group's higher PPL (Figure 3) and increasing TPL (Figure 3) suggest impaired learning and memory. Although the cigarette group's PPL started to decrease after the second day and continued until the fifth day, the decrease was not significant (p=0.8), indicating impaired memory. The sharp increase in TPL on Day 3 (D3) indicates hyperactivity and a motivation to explore the maze further after locating the target hole.

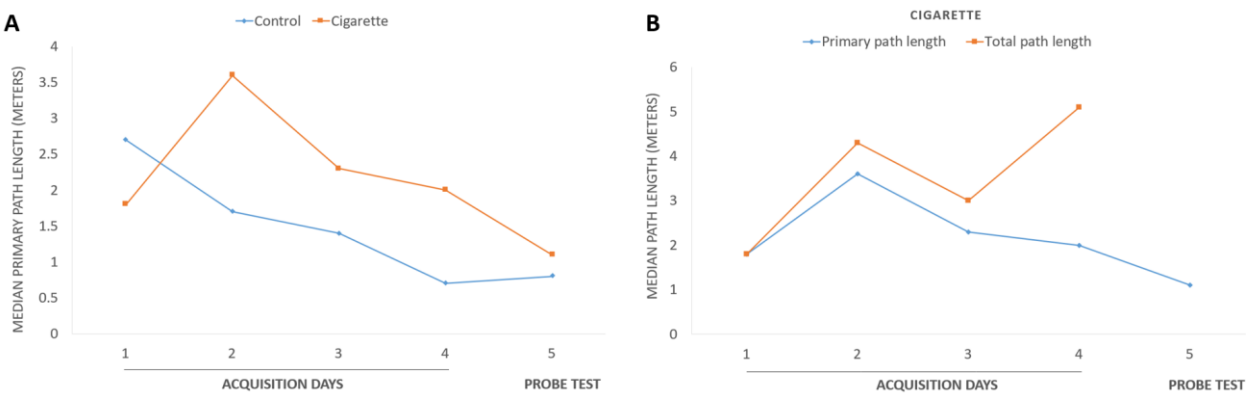

Figure 3: Changes in the primary path length to locate the target hole (A) and variations in the primary and total path lengths in the Cigarette Group (B)

A: Control: Friedman test indicates no significant [$X^2(4) = 7.848$, $p=0.097$] decrease in the primary path length. Cigarette: There was no significant [$X^2(4) = 1.600$, $p=0.809$] difference between the daily changes in the primary path lengths. $n=8$, $p \leq 0.05$.

Errors: The number of primary errors, which refers to the head deflections into incorrect holes before locating the target hole, significantly decreased in both the control ($p=0.001$) and cigarette groups ($p=0.001$) over the acquisition period (Figure 4). Significant differences were observed between Day 1 and Day 3 for the control group and between Day 2 and Day 5 for the cigarette group.

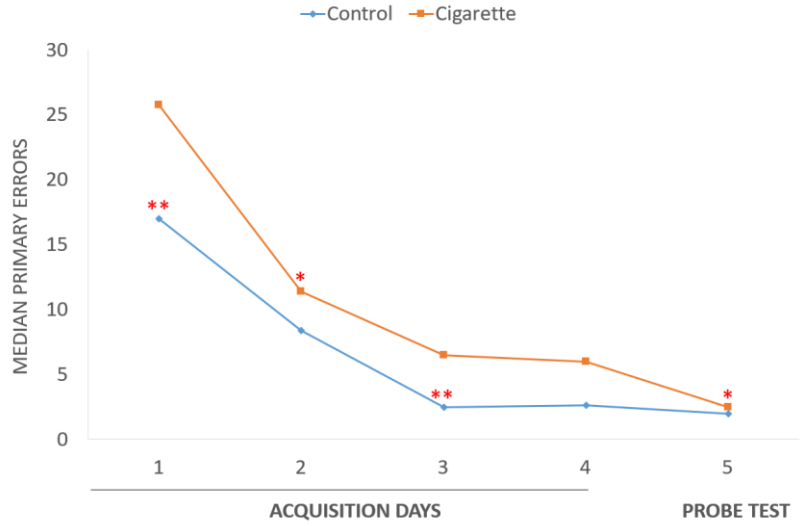

Figure 4: Changes in the number of primary errors made before locating the target hole.

Control: Friedman test indicates significant decrease in the errors [$X^2(4) = 22.405$, $p=0.000$] between D1 and D3 ($p=0.009$) after post hoc comparison. Cigarette: Friedman Test indicates significant [$X^2(4) = 17.700$, $p=0.001$] decrease in the errors that occurred between D2 and D5 ($p=0.003$) after post hoc comparison. $n=8$, $p \leq 0.05$.

Preferences for Target Hole: On the probe test, mice in both groups showed a preference for the virtual target hole (Figure 5).

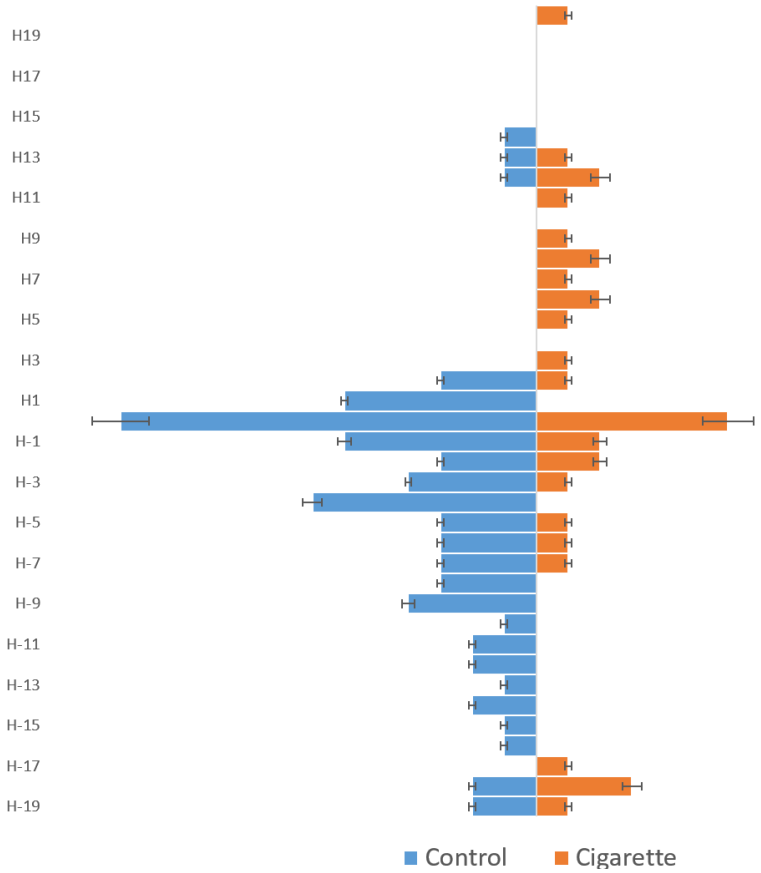

Figure 5: Higher preference for the virtual target hole in the control and cigarette groups on the probe test day (D5).

Mann-Whitney U-test, there was no significant ($p=0.102$) difference in the preference of the virtual target hole between the control and the cigarette group. $n=8$, $p \leq 0.05$, * indicates statistical significance, and its absence indicates insignificance.

Search Strategy: On the probe test day (D5), mice in the cigarette smoke-exposed group predominantly used the "mixed" search strategy, whereas mice in the control group preferred the "direct" strategy (Figures 6). The cigarette group's abnormal preference for the mixed strategy, instead of the direct strategy, indicates difficulties in learning and recalling the target hole's location. These mice often had to search serially through several holes before accidentally finding the target hole (Figure 6).

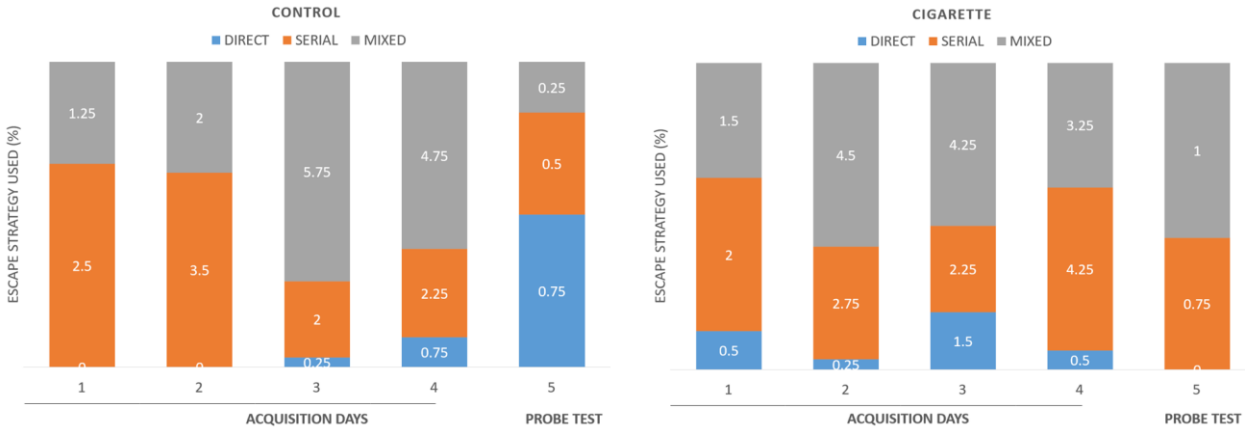

Figure 6: The preferred escape strategy used by mice to locate the target hole in the control and mosquito coil groups.

DISCUSSION

Although cigarette smoke was not associated with significant weight loss; however, from the literature, exposure to cigarette smoke during the third trimester was a significant and independent predictor of lower birth weight percentile in newborns (Dessi *et al.,* 2018). Moreover, routine air pollution has been linked to increased infant mortality and the development of bronchopulmonary diseases, particularly in children (Korten *et al.,* 2017). Rats exposed to low concentrations of CO prenatally, showed lower birth weights and other neurodevelopmental problems (Omotoso *et al.,* 2018; Nemoto *et al.,* 2021).

The study monitored environmental temperatures to ensure consistent conditions between control and experimental groups, aside from CO exposure. Results showed no significant temperature difference between the gas chamber during exposure and the ambient environment of the control groups. Thus, it was concluded that consistent temperature conditions were maintained, and behavioral changes observed were not due to temperature variations.

The CO exposure dose, measured by the PCMM05 Pyle CO gas analyzer, did not correlate well with the %COHb as predicted by the Coburn, Forster, & Kane, (1965) CFK model. However, the %COHb was significantly higher in the cigarette group compared to the control. From the literature, individuals mildly exposed to CO (<1 %COHb) often retain memory functions comparable to control groups, and sometimes excel in areas such as learning and word recall, as measured by Buschke's verbal memory test. Attention span was also found to be better, with shorter visual reaction times compared to controls (Deschamps *et al.,* 2003). Although our control group's mean %COHb was up to 2%, it remained within the non-smoker range and posed minimal toxicity risk. Such mild exposure was even found to enhance learning (Kurawa *et al.,* 2020).

The balance beam test was used to screen all mice for motor coordination deficits before the study began (Brooks and Dunnett, 2009). In the Barnes maze (BM), parameters like primary latency (PL), primary error (PE), primary path length (PPL), and search strategies were analyzed to assess spatial learning and memory (O'Leary and Brown, 2013). Effective learning was indicated by reduced PL, PPL, and PE over four days, with the lowest values on the probe test day (D5) and a preference for direct search strategies (Harrison *et al.,* 2009). These tests reliably detect subtle neurological dysfunctions from CO poisoning (Sunyer *et al.,* 2007).

The control group exhibited expected learning and memory characteristics, with significant decreases in primary latency (PL) and primary error (PE) during the acquisition period, mastering the maze by day three. On day five, the virtual target hole had the most pokes, indicating excellent recall. Rapid strategy shifts by day two demonstrated superior learning abilities.

The cigarette group however, showed an insignificant decrease in primary latency (PL) but a significant reduction in primary error (PE) over the acquisition days. They preferred the virtual target hole (VTH) on day five (D5) and mainly used the "serial" search strategy. In our environments, exposure to CO from passive cigarette smoke is almost unavoidable. The emergence of shisha smoking is now considered the second global tobacco epidemic after cigarettes (Maziak, 2011). Levels of COHb as high as 24% have been associated with Narghile (water pipe, hookah, shisha, goza, hubble bubble, and argeela) smokers (La Fauci *et al.*, 2012). Shisha smoking has become a prevalent method of tobacco use among youth (Blank *et al.,*

2011). Arziman *et al.* (2011) reported about 5 cases of CO poisoning following narghile (shisha) smoking.

The cigarette group exhibited delayed learning and memory acquisition and increased activity, indicated by longer total path length. Nicotine, a central nervous system stimulant, likely caused hyperactivity, alertness, and possibly euphoria, affecting performance. On average, a cigarette delivers up to 2 mg of nicotine, a dose that is stimulatory, though higher doses (50 – 100 mg) can be harmful. Nicotine stimulates nicotinic acetylcholine receptors and promotes the release of neurotransmitters, enhancing alertness and euphoria, contributing to addiction (Moreland-Capuia and Moreland-Capuia, 2019).

The neurotoxicity of cigarette smoke may stem from its over 7,000 chemicals, many of which can damage neurons (Rodgman and Perfetti, 2013; CDC, 2022). This damage often occurs through oxidative stress and inflammation (Hussain and Ekhzaimy, 2022), neurotransmitter modulation, and nicotine's stimulatory effects on the brain (Benowitz, 2010). While nicotine might acutely enhance attention and memory (Heishman *et al.,* 2010), chronic exposure leads to downregulation of receptor sensitivity and long-term cognitive deficits (Valentine and Sofuoglu, 2018). Disruptions in neurotransmitter systems, especially those involving dopamine and acetylcholine, are crucial since these systems play significant roles in learning and memory (Xu *et al.,* 2019). Chronic smoking has been linked to reduced brain volumes in critical areas like the hippocampus and prefrontal cortex, which are vital for processing and retaining spatial information (Durazzo *et al.,* 2012). Both animal models (Zhang *et al.,* 2018) and human studies (Debette and Markus, 2010; Sabia *et al.,* 2012) consistently show that smoking impairs performance in tasks requiring spatial memory. Long-term consequences for smokers include accelerated cognitive decline and higher risk for neurodegenerative diseases, which further exacerbate memory impairments (Wu *et al.,* 2020). Although there is potential for recovery of cognitive functions after smoking cessation, the extent and pace of recovery depend on various factors, including the duration of smoking and overall health.

CONCLUSION

This study explored the impact of CO gas exposure from cigarette smoke on body weight and spatial memory in mice. The dose and duration of the cigarette smoke used in this study did not significantly affect body weight. However, mice exposed to cigarette smoke had blood carboxyhemoglobin (%COHb) levels well above WHO safety limits. While the control group exhibited strong learning and memory skills, the cigarette smoke-exposed group showed delays in learning and memory abilities. The nicotine in cigarette smoke probably induced hyperactivity and altered learning abilities, potentially offsetting the cognitive deficits caused by CO exposure. Further research is warranted to explore the long-term effects and underlying mechanisms of cigarette smoke on cognitive function.

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