UNIVERSITY OF CALIFORNIA SAN DIEGO

Chronic Nicotine Exposure Leads to Neurobehavioral Changes in CCI-induced TBI Mouse Model

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

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The Thesis of Dongsheng Wang is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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TABLES OF CONTENTS

LIST OF FIGURES

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The current thesis contains materials being prepared for publication. Mr. Wang was the principal author of this thesis.

ABSTRACT OF THE THESIS

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Traumatic brain injury (TBI) causes detrimental neurological conditions resulting from neuronal loss, damage to the blood-brain barrier (BBB), and secondary injury due to chronic neuroinflammation. Evidence shows that chronic tobacco use may exacerbate TBI neuropathology. The active component of tobacco, nicotine, activates neuronal nicotinic acetylcholine receptors (nAchRs). Caveolin-1 (Cav-1), a cholesterol-binding and scaffolding

protein, localizes to plasmalemmal membrane lipid rafts (MLRs) and regulates nAchRs expression. Previous studies demonstrated that TBI alters MLRs and decreases Cav-1 and nAchR expression. However, it remains unknown if chronic nicotine exposure would aggravate TBI-induced neurobehavioral deficits. In this current study, we aim to elucidate the effects of chronic nicotine vaping and TBI on nAchRs expression and associated biochemical and behavioral changes. We used a murine controlled cortical impact (CCI) model to induce TBI. CCI mice were then subjected to vapor exposure for 6 weeks. Multiple behavioral tests were utilized to evaluate post-exposure changes in motor functions, social motivation, and object recognition memory. Immunoblot assays were used to assess nAchRs expression. Our findings demonstrated that chronic nicotine exposure inhibited social motivation in CCI mice. Recognition memory impairment was also observed due to TBI and chronic vaping. Biochemically, we observed decreased hippocampal β2-containing nAchRs in vapor-exposed Sham surgery and CCI mice; however, chronic nicotine alone did not alter Cav-1 expression. Overall, our findings suggest that the loss of β2-containing nAchRs due to chronic exposure to E-cigarette vapor may exacerbate CCI-induced behavioral deficits.

INTRODUCTION

Traumatic brain injury (TBI) is one of the leading causes of disability and death in the United States. According to the Centers for Disease Control and Prevention (CDC), 223,135 TBI-related hospitalizations were documented in 2019. In 2020, 64,362 deaths were attributed to TBI. Although TBI generally poses a greater risk to people who are 75 years and older, the mortality rate of younger patients was also significantly higher compared to their age and sex-matched controls [1]. The primary cause of TBI is a strong and abrupt force that deforms and damages the brain. Hemorrhage, tissue damage, and corruption of the blood-brain barrier (BBB) are common conditions observed immediately after the injury. In addition to the initial injury, TBI patients' outcomes can be affected by secondary injuries, which include chronic inflammation, excitotoxicity, neuronal apoptosis, and impairment of the BBB [2]. Our lab has previously demonstrated that TBI disrupts membrane/lipid rafts (MLRs), lipid-enriched microdomains that cluster and modulate key receptor signaling components necessary for synaptic function (e.g. neurotrophic and neurotransmitter receptors) [2,3].

The nicotinic acetylcholine receptors (AchR), which bind to the neurotransmitter acetylcholine (Ach), localize to MLRs. Lipid extraction and depletion experiments have demonstrated that the structural integrity of MLRs is essential in maintaining the subcellular localization of nAchRs [4,5]. Biochemically, acetylcholine is responsible for mediating the actions of the cholinergic system. A variety of important cognitive functions, such as memory, learning, and sensory processing, can all be modulated by the cholinergic system. Cholinergic actions have also been shown to regulate mood and reward-related behaviors [6,7,8]. Nicotine, a natural chemical found in tobacco leaves and the active ingredient in cigarettes, is shown to

selectively activate neuronal nicotinic acetylcholine receptors. An epidemic of nicotine abuse has been observed in recent years as electronic cigarettes (E-cig) started gaining increased popularity among adolescents and adults. Despite containing fewer toxic chemicals than traditional tobacco smoke, the high nicotine content and heavy metals in E-cig aerosol could still pose serious health hazards to smokers [9,10]. Additionally, chronic exposure to nicotine has been shown to lead to long-lasting changes in the conformational states of nAchRs. For example, Eilers and colleagues have demonstrated that prolonged exposure to low-dose nicotine can significantly decrease calcium influx mediated by activated α4β2 receptors [11].

Chronic exposure to nicotine has been shown to induce paradoxical effects on the physiology and cognitive functions of smokers [12]. While some reported that chronic nicotine dependence leads to elevated anxiety [13], continuous nicotine exposure at low dosage was shown to enhance cognitive performance and alleviate schizophrenic symptoms [14,15]. Since both TBI and chronic nicotine exposure can alter the cholinergic signaling pathway, this current study aimed to assess the comorbid effects of TBI and chronic vaping on brain biochemistry and associated behavioral effects.

Method and Material

Animals

All mice (C57BL/6J; The Jackson Laboratory, Bar Harbor, ME, USA) were treated in compliance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, Bethesda, MD, USA). All animal use protocols (#20-021) were approved by the Veterans Administration San Diego Healthcare System Institutional Animal Care and Use Committee (IACUC) before procedures were performed. Mice were housed under normal conditions with a sufficient supply of water and food.

Experimental Design

C57BL/6J male mice were randomly divided into 4 groups: Sham-vehicle, Sham-nicotine, CCI-vehicle, and CCI-nicotine. At 3 months of age**,** mice underwent either Sham surgery or CCI. The baseline assessment for social preference (SP) was taken one-week post-surgery followed by vehicle or nicotine exposure for 6 weeks. One week post vape exposure, nestling test, social interaction test, novel object recognition test, and open field test were performed. At the conclusion of all behavior testing, mice were euthanized, and tissue was assessed for biochemical and histological changes.

Controlled Cortical Impacts (CCI) Model

CCI procedure was performed as described previously [2]. Mice were anesthetized with isoflurane (5% induction for 30s followed by 1.5% maintenance mixed with oxygen at 1 L/min). Anesthetized mice were stabilized in a stereotaxic frame by ear bars. As previously described, a small cranial window was created on the right hemisphere using a portable drill. After carefully removing the bone flap, a 3-mm-diameter stereotaxic impactor (Impact one; myNeuroLab.com, Richmond, IL, USA) was centered on the dura mater. The impactor was then accelerated to 3 m/s with the impact depth set to 1 mm below the cortical surface. A sterilized cover glass was placed over the cranial window to shield brain tissues from infection. Tissue glue and surgical clips were used to close the incision site. Animals were kept on a 37 ºC heating pad throughout the duration of the operation. The animals were allowed to recover for one week before vaping exposure.

Vapor Exposure Chamber Model

Stick V9 Max vape pen kit was used to aerosolize nicotine-containing E-liquid. The stick battery provides an output voltage of 2.1V to 4.1V and an output power of 60W. An E-liquid tank of 8.5 ml volume capacity is attached to the stick battery. The E-liquid vehicle contains 70% VG and 30%PG. Nicotine is added to the vehicle solution to achieve a concentration of 24 mg per ml of vehicle. A paired Baby V2 S2 0.15 Ohm coil is used to create intense clouds of vape smoke. The mouthpiece of the vape pen is directly connected to the exposure chamber via PVC and metal tubings for effective delivery of vape smoke. A vacuum pump set at 50 psi is used to provide ventilation and draw in E-cig vapor into the exposure chamber. Heating tapes are secured around the walls of the exposure chamber for temperature control. A temperature probe

is inserted into the chamber to provide constant readouts. A single exposure session lasts 40 minutes, and each puff cycle includes 5 s of heated e-cig vapor followed by 25 s of air.

Determination of optimal temperature and exposure duration

Previous studies have shown that chamber temperature could affect the size of aerosolized particles and thus smoke delivery efficiency [7]; we thus tested the effect of varying chamber temperatures on the efficiency of vape smoke delivery. Mice were exposed to vehicle vapor containing Evans Blue (EB) fluorescent dye at four different temperatures (21, 28, 32, 36 °C) within the animal's thermal neutral zone. Animals were euthanized immediately after exposure. The whole lungs, along with the trachea, were carefully dissected and inflated and air-dried overnight. The fluorescent signal was visualized by IVIS Spectrum In Vivo Imaging machine (level = high, Em = 700, Ex = 640, Epi-illumination, Bin:(M)8, FOC:13.2, f2, 15s) was used to detect fluorescent signals in the lungs.

Next, time-dependent exposure studies were performed to determine the optimal exposure duration. Mice were exposed to vehicle + nicotine for 5, 20, 40, and 60 minutes respectively. Blood and lung tissues were collected immediately at the end of each exposure time point. The cotinine ELISA kit was used to quantify cotinine concentration in blood plasma and lung tissue homogenates.

Behavioral Tests

Social Approach (SA)

Mental illness and social anxiety disorders are commonly seen in individuals suffering from chronic TBI[16]. The social preference was assessed before and after vape smoke exposure to evaluate the potential effects of nicotine exposure on the CCI mice [17]. The social approach (SA) test consists of a habituation stage followed by a testing stage. At the habituation phase, the test mouse was first given 10 minutes to explore the three-chambered arena. At the testing stage, the test mouse will be given another 10 minutes to explore the arena in the presence of a stranger mouse and an object. The time spent with the object and the stranger mouse was recorded for the entire duration of the test. Pre-pubescent male mice were used as partners for the social interaction test. The sociability of the animals was evaluated by the social preference index defined by the difference between the percentage of time spent with a stranger mouse over an inanimate object.

Open Field (OF)

The Open Field (OF) test was performed to assess mice's general locomotion and exploratory behavior [3]. Mice were placed in a square arena (41 x 41 x 34 cm enclosures) illuminated by a bright light. A computerized video tracking system (Noldus XT 7.1, Leesburg, VA, USA) was used to record and analyze their activities during a 10-minute test session. Distance moved (cm), velocity (cm/s), and time spent in the center of the arena (s) were recorded to evaluate general and exploratory activity as previously described.

Nestling

To assess the effect of nicotine on CCI-induced motor deficit, nestling, an intrinsic behavior performed by rodents that requires fine motor skills was assessed as described by Deacon (2006) [18]. Each Individual mouse was separated and placed in single cages approximately 1 hr before dark. Nestlets are weighed and standardized to 3.0 g each. Except for supplied nestlets, all enrichment in the cage was removed. Nests were scored on a scale from 1 to 5 the next day. (1: >90% of untouched nestlets; 2: 50–90% of nestlet intact; 3: 10–50% of nestlet intact but the nest is not identifiable; 4: <10% of nestlet intact, nest is identifiable but flat; 5: <10% of nestlet intact, nest is identifiable with high walls).

Novel Object Recognition (NOR)

To assess the effect of chronic nicotine exposure on CCI-induced cognition deficit, we performed novel object recognition (NOR) test, a fast and sensitive test to detect recognition memory changes [19]. NOR consists of three stages: habituation, object familiarization, and novel object recognition. On the first two days, test mice will habituate in the test arena (dark Plexiglas, 25 x 35 x 25 cm) one at a time for 5-30 minutes. On the third day, the animal is allowed to habituate to the test room for 30 minutes. They are then presented with two similar objects for 30 minutes. Mice are then removed from the test arena for an inter-trial interval of 24 hours before they are put back in the arena and exposed to one familiar and one novel object for 2 - 30 minutes. A video tracking system was used to record the animal's interactions with the objects. The discrimination index (DI), which s calculated by dividing the difference between time spent with a novel object and a familiar object over the sum of time spent with both objects.

Immunoblot (IB)

Freshly dissected hippocampi were homogenized in cold 500 mM sodium carbonate buffer (pH 11.0; Protease and phosphatase inhibitor cocktail was added) and sonicated three times for 10s. Bradford assay was performed to standardize sample concentration to 1.0 ug/ul. Tissue homogenates were then immunoblotted (IB) with primary antibodies for Cav-1 (Cell signaling #3267; 1:1000), GAPDH (Cell Signaling #2118S; 1:1000), nAchR α7 (Millipore Sigma AB15332; 1:500), and nAchR β2 (Novus Biologicals NBP1-28467; 1:500), TrkB (BD Biosciences 610102; 1:1000). HRP-linked anti-rabbit IgG (Cell Signaling #7074S, 1:1000) was used for secondary incubation. All band intensity was obtained using Photoshop. GAPDH intensity was used for normalization.

Biochemical assessment of MLRs

Whole-cell lysates were subjected to sucrose gradient fractionation as previously described [2]. 183 ul of whole-cell homogenate was mixed with an equal volume of 80% sucrose dissolved in MBS buffer (25 mM MES, 150 mM NaCl, and 2 mM EDTA). 1.1 ml of 35% sucrose solution and 732 ul of 5% sucrose solution were sequentially layered on top of the 40% lysate and sucrose mixture. The sucrose gradient was then centrifuged with a bench-top ultra-centrifuge using an SW-41 motor at 39000 rpm for 17 hours at 4°C. The first three fractions (183 ul each) were discarded, and only fractions 4-12 were collected for experiments. MLRs are

present in buoyant fractions 4-6 based on their biophysical properties. Fractions 4-12 were then subject to IB.

Cotinine ELISA

The Cotinine Direct ELISA kit (Calbiotech, CO096D) was used to detect cotinine concentration in blood plasma and lung tissue homogenates. Blood was collected using containing EDTA-containing tubes to prevent coagulation. Plasma was collected via high-speed centrifugation of whole blood sample at 13k r.p.m. for 10 minutes at 4 ºC. Lung tissues were homogenized quickly in cold PBS and adjusted to uniform concentration by Bradford assay. Cotinine concentration in blood plasma and lung tissue homogenates was assessed as instructed by the provided protocol.

Result

Assessment of exposure efficacy for the vapor exposure chamber

To determine the optimal exposure temperature for our exposure protocol, we first evaluated smoke vapor delivery efficiency at four varying temperatures. Fluorescent imaging of inflated lungs detected no significant difference in EB intensity at all four selected temperatures after 30 minutes of exposure (**Supplemental Figure 1**). In consideration of the ambient temperature (25 ºC) and the animal's thermal neutral zone, 28 ºC was chosen as the exposure chamber (**Figure 1A**) temperature for all following exposure sessions.

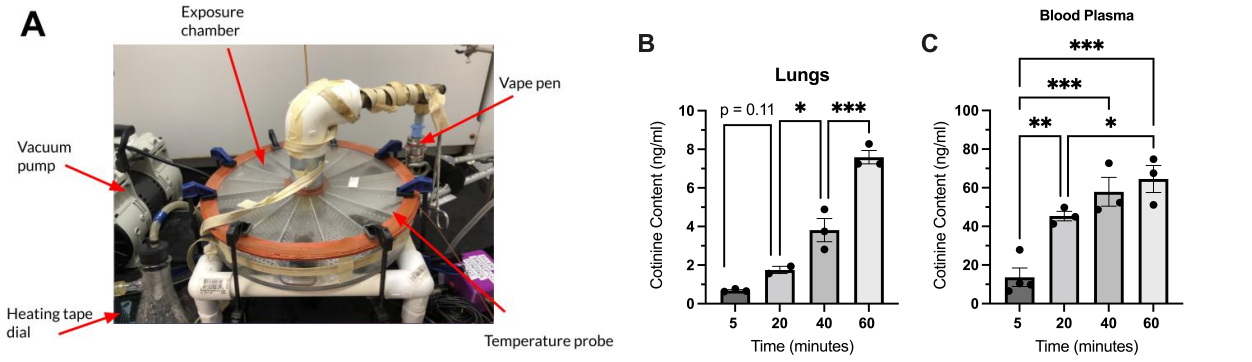


Figure 1. Assessment of nicotine delivery efficacy via a new vapor exposure system. (A) Components of the vape smoke exposure chamber. **(B-C)** Cotinine concentrations in lung tissue homogenates and blood plasma. Cotinine concentrations were analyzed using a one-way analysis of variance by Fisher's LSD test, n=3 per group. Significance was assumed when $p < 0.05$. *$p < 0.05$, **$p < 0.01$, *** < 0.001.

To determine optimal exposure duration, cotinine concentration was quantified in plasma after 5, 20, 40, and 60 minutes of exposure. As shown in **Figure 1B**, plasma cotinine level significantly increased after 5 minutes, reaching a peak level at 40 minutes. No significant difference was found between 40 min and 60 min exposure groups. We further measured cotinine

concentration in lung tissue which exhibited a similar trend as observed in plasma (**Figure 1C**). The steadily increasing cotinine concentration in plasma and lung homogenates confirmed the efficacy of our exposure chamber in delivering nicotine-containing vapor. Considering that the plasma cotinine doesn't increase further after 40 minutes of exposure, 40 min is chosen as the exposure duration in the following experiments.

Chronic nicotine exposure increases nestling but decreases sociability in CCI mice

The nestling test is a widely used behavioral test that evaluates rodents' motivation and motor skills for nest-building, a highly species-typical behavior. More importantly, nestling assessment is sensitive to damage to multiple brain regions, including the pre-optic area, septum, and hippocampus [18]. Thus, nestling was performed after exposure. No difference in nestling score was observed between the Sham-Vehicle group and the Sham-Nicotine group (**Figure 2A**), suggesting that chronic nicotine exposure alone does not alter the animal's tendency and ability to build nests. CCI-Vehicle group exhibited a significant decrease in nestling score compared to the Sham-Vehicle group, indicating deficits in fine motor skills due to brain trauma. Interestingly, mice in CCI -Nicotine group demonstrated a nonsignificant increasing trend in nestling score compared to the CCI-Vehicle group ($p = 0.06$).

TBI and chronic nicotine use strongly correlate with increased susceptibility to depression, personality disturbances, and anxiety-like behaviors caused by multifaceted biochemical, morphological, and structural alterations in the central nervous system (CNS) [22,23]. SA test was thus performed to evaluate sociability [17]. As shown in **Figure 2B**, prior exposure, all four groups of mice exhibited a similar level of preference for a stranger mouse

over an inanimate object, indicating no effect from CCI alone on sociability. After 6 weeks of chronic vape exposure, mice from the CCI-Nicotine group showed a significant decrease in preference for social interactions ($p = 0.017$), while the Sham-Nicotine group only exhibited a nonsignificant decrease in preference index, suggesting that chronic nicotine exposure after TBI significantly impacts the animal's social motivation. As shown in **Figure 2C**, no difference in

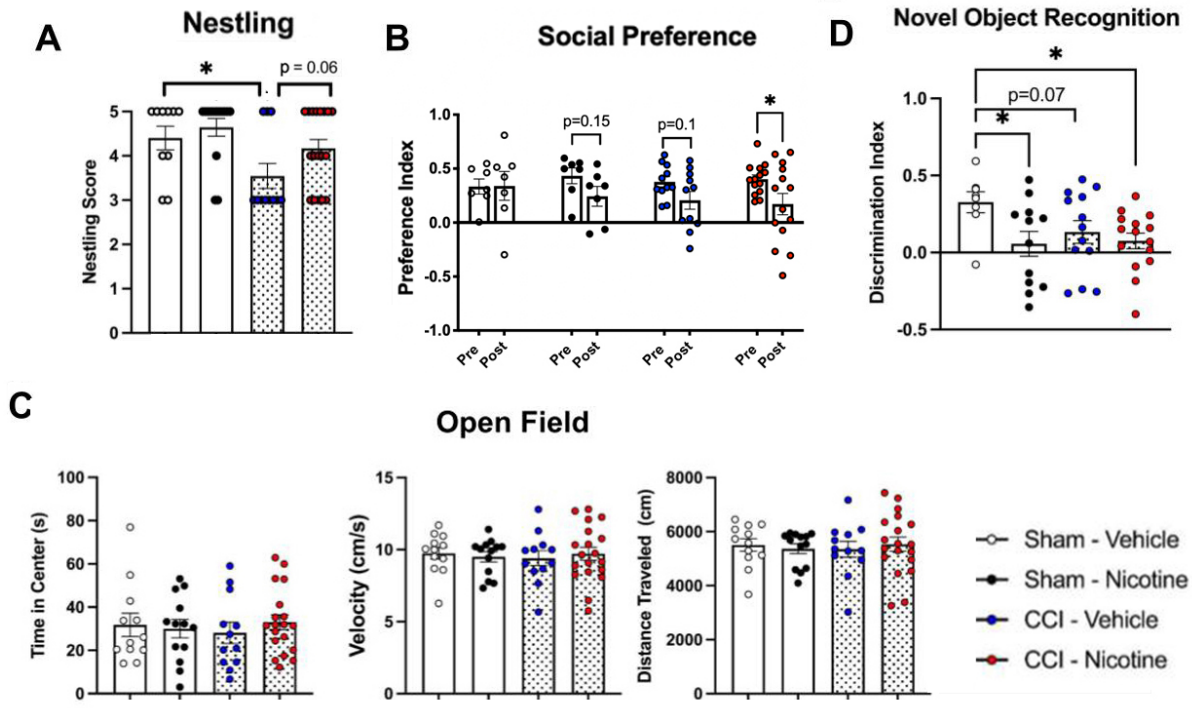


Figure 2. Chronic nicotine exposure and CCI impair object recognition memory, and inhibit social motivation. (**A**) Nestling test. (**B**) Social approach test result was presented as preference index at both pre- and post exposure timepoints. (**C**) Open field test. No significant difference was found in time spent in center, velocity, and total distance traveled. (**D**) Recognition memory was presented as discrimination index. Nestling, NOR, and OF data were analyzed using one-way analysis of variance (ANOVA) by Fisher's LSD multiple comparison tests. Social approach were analyzed using two-way ANOVA by Fisher's LSD tests. Significance was assumed when $p < 0.05$. *$p < 0.05$.

total moving distance and velocity was observed among all four groups. Furthermore, no significant difference was observed in time spent in the center of the arena, indicating a similar

level of anxiety and exploratory behavior among all four groups. This result also confirmed that the decreased social preference observed in the CCI-Nicotine group was not due to altered baseline anxiety or decreased exploratory behavior, but was specific to social motivation.

Chronic nicotine exposure and CCI alone impair NOR

The cholinergic system has been shown to mediate several essential cognitive processes like arousal, attentional control, and memory retrieval [24,25]. We thus measured NOR to evaluate animals' recognition memory after CCI and chronic nicotine exposure. Sham-Nic mice exhibited a significant decrease in discrimination index compared to the Sham-Vehicle group, suggesting deficits in recognition memory due to chronic exposure to nicotine alone (**Figure 2D**). Mice from CCI-Vehicle and CCI-Nicotine groups exhibited decreased discrimination index resulting from CCI injury in the hippocampal region; however, no significant difference was measured within the two CCI groups ($p=0.5259$, One-Way ANOVA), suggesting that nicotine did not exacerbate CCI-mediated memory deficits.

TBI and Chronic exposure to nicotine lead to the loss of β2-containing nicotinic acetylcholine receptors in the ipsilateral hippocampus.

α7 and α4β2 nAchRs are two functionally important acetylcholine receptors predominantly expressed in the hippocampus; thus, we assessed the effects of CCI and chronic nicotine exposure on the expression of α7 and β2 receptor subunits. No significant difference was observed in α7 nAchRs expression among all four groups although α7 nAchRs expression in the CCI-Nic group exhibited a non-significant decreasing trend compared to the Sham-Nicotine

group (p = 0.059). IB of β2 nAchRs revealed a significant decrease in Sham-Nicotine, CCI-Veh, and CCI-Nicotine groups compared to the Sham-Nicotine group (**Figure 3A&B**). Taken together, our data indicate that CCI and chronic nicotine exposure may independently decrease the expression of β2 nAchRs.

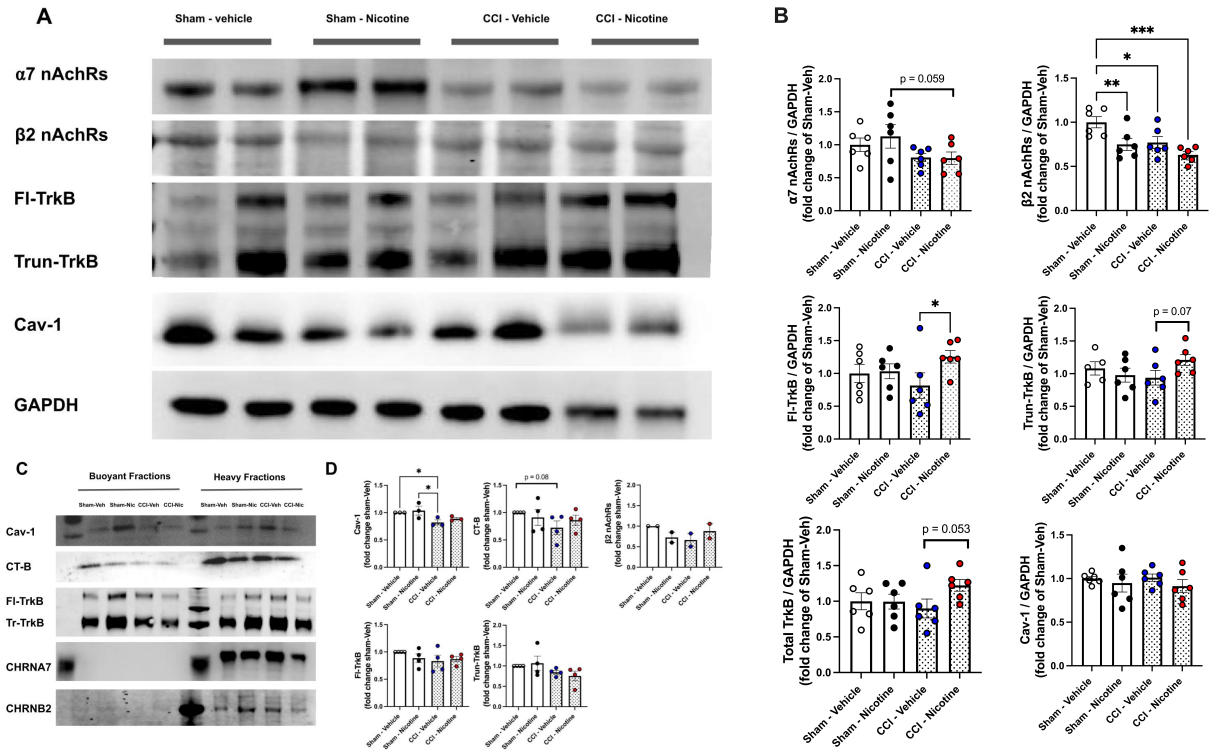


Figure 3. Post-exposure biochemical characterization of the ipsilateral hippocampus. (**A-B**) IB revealed decreased β2-containing nAchRs in Sham-Nicotine, CCI-Vehicle, and CCI-Nicotine mice. An upregulation of fl-TrkB receptor was detected in CCI-Nicotine group. IB (**C-D**) of buoyant fractions displayed decreased MLR-associated Cav-1. All IB results were analyzed using one-way ANOVA by Fisher's LSD test, n=6 per group. Significance was assumed when $p < 0.05$. *$p < 0.05$, **$p < 0.01$, *** < 0.001.

Tropomyosin receptor kinase B (TrkB) is an indispensable membrane receptor that mediates critical biological processes such as neuronal survival, axonal growth, and cell proliferation via TrkB/BDNF signaling pathway[26]. We previously showed down-regulated whole-cell and MLR-associated TrkB expression in hippocampal tissues from mice with acute

brain injuries [2,3,27]. In this current study, an increased fl-TrkB expression was observed in the CCI-Nicotine group compared to mice from the CCI-Nicotine group (**Figure 3C**); however, no difference was observed within the Sham surgery groups. Additionally, IB revealed no difference in truncated and total TrkB among all four groups, suggesting that chronic nicotine exposure may enhance TrkB signaling in CCI mice by upregulating the expression of fl-TrkB receptors.

IB of MLRs revealed decreased cholera toxin subunit-B labeling ($p=0.08$) and Cav-1 expression in the CCI-Vehicle group compared to the Sham-Vehicle group, indicating MLR disruption resulted from CCI damage (**Figure 3C&D**). This result is consistent with previously published work from our group [27]. A nonsignificant decreasing trend in MLR-associated Cav-1 expression in CCI-Nicotine was also observed compared to the Sham-Vehicle group ($p = 0.0634$). However, MLR-associated Cav-1 expression was unaffected by chronic exposure to nicotine. Lastly, our preliminary data on MLR-associated β2 nAchRs expression displayed a decreasing trend in Sham-Nicotine and CCI-Vehicle groups compared to the Sham-Vehicle group.

Discussion:

The present study demonstrated the combination of chronic nicotine exposure and CCI induced significant neurobehavioral changes. Specifically, chronic nicotine vaping inhibited social motivation in CCI mice. Furthermore, chronic nicotine exposure and CCI were shown to independently impair NOR memory, but when combined, NOR memory deficit was not worsened. Biochemical analysis revealed that chronic nicotine exposure decreased hippocampal β2 nAchRs expression in CCI mice, which may in part contribute to the decreased social activity found in these groups. While CCI decreased MLR-associated Cav-1, chronic nicotine didn't affect MLR-associated Cav-1 expression in the Sham or CCI group.

To study the effects of nicotine on various physiological systems, many labs have heavily relied on intraperitoneal injection or oral consumption for nicotine delivery [28]. However, such traditional delivery approaches may not accurately reflect human physiology after exposure to nicotine [28,29]. With our semi-automated vapor exposure chamber, we are capable of mimicking human vaping sessions while easily controlling chamber temperatures and exposure duration. In this current study, we have successfully demonstrated the efficacy of our exposure chamber in nicotine delivery by confirming the presence of cotinine, a predominant metabolite of nicotine, in the blood plasma. Additionally, nicotine deposition was detected in vapor-exposed lungs as in human cigarette users. Thus, our vapor exposure chamber is proven to provide a more physiologically and clinically relevant method for nicotine administration.

Chronic tobacco use has been associated with increased susceptibility to psychological disorders, including depression, anxiety disorders, and mood regulation disorders. TBI has been reported to cause impaired social perspective-taking abilities and increased anxiety [22,23,30]. In the current study, we observed a significant inhibition of social activity in nicotine-exposed CCI mice during the social approach test. To determine whether this decreased social activity is due to decreased social motivation, artifacts from increased anxiety, or decreased locomotion, we performed the open field test. Our result showed that all mice spent a similar amount of time in the center area of the open arena and exhibited similar moving speed. Thus, we concluded that the inhibited social approach behavior is specifically due to decreased social motivation. This result is similar to findings from another group that reported direct nicotine infusion into the dorsal hippocampus reduced interaction time between the test mouse and the partner mouse [31]. Considering that the CCI model utilized by our lab causes extensive damage and tissue loss to the motor cortex and the hippocampus, we subsequently performed IB analysis for the ipsilateral hippocampal tissue. Our result revealed that both CCI and chronic nicotine exposure reduced the expression of β2-containing nAchRs. Interestingly, Zoli and colleagues reported that β2 nAchRs knockout mice displayed accelerated cognitive decline due to increased neuronal loss and neuroinflammation during ageing [32]. Thus, it is plausible that the loss of β2 nAchRs, due to the combined effects of CCI and chronic nicotine exposure, contributed to decreased sociability in CCI-Nicotine mice. We also observed a decreasing trend in the social preference index after chronic nicotine exposure in the Sham group. Dopaminergic neurons in the ventral segmental area (VTA) and the nucleus accumbens (NAc), and neurons in the medial prefrontal cortex (mPFC) all express nAchRs that can be activated by acetylcholine and nicotine, and the VTA,

NAc, and mPFC form the mesolimbic system which regulates approach and avoidant behaviors in mice [33,34]. Thus, aside from TBI-induced brain trauma, chronic nicotine exposure could potentially alter sociability in mice by interfering with the mesolimbic neural network. In line with these previous findings, we discovered that chronic nicotine exposure alone decreased β2-containing nAchRs expression in the ipsilateral hippocampus

While chronic nicotine exposure has been shown to promote cognition, nicotine withdrawal symptoms have been shown to produce deficits in cognitive functions, including sustained attention, working memory, and response inhibition [35,36]. The current study detected impaired NOR memory in Sham surgery mice approximately 3 weeks after the 30-day nicotine exposure period, a result in line with other studies [37]. Esaki and colleagues recently reported that the activation of α7 nAchRs and α4β2 nAchRs could lead to enhanced object recognition performance [38]. In line with this previous finding, our current study revealed decreased expression of β2-containing nAchRs in the Sham-Nicotine group, suggesting that the loss of hippocampal β2-containing nAchRs may contribute to impaired NOR memory. The cholinergic circuit, which consists of the medial septum (MS), ventral diagonal band of Broca (vDB), and the nucleus basalis of Meynerts (nBM), is known to regulate several essential cognitive functions. Loss of cholinergic cells due to neurodegenerative diseases was shown to severely affect attentional control, learning, and memory-related tasks [39]. In this current study, we also observed impaired object recognition memory in the CCI mice compared to the Sham-Vehicle group, consistent with what is observed in TBI patients [40,41]. The CCI model utilized in the current study causes severe damage to the cortex and the hippocampus, both receiving projections from cholinergic neurons MS, the vDB, and the nBM [42]. Thus, the deficit in NOR

memory observed in CCI-Vehicle mice possibly reflects a disrupted cholinergic neural network due to brain trauma. No difference in NOR memory was detected between vehicle-exposed and nicotine-exposed CCI mice. we suspect that the NOR test may not be sensitive enough to further differentiate nicotine-induced NOR memory deficit from trauma-related cognitive impairment.

Lesions to the hippocampus have been previously shown to produce poorer nestling outcomes in WT mice [43]; thus, the decreased nestling score observed in CCI mice is likely due to the damaged motor cortex and the hippocampus. Importantly, unaffected OF test data suggest that the decreased nestling score in CCI mice is not due to inactivity or a general decrease in locomotion. Unexpectedly, a nonsignificant improvement in nest-building was observed in CCI mice after chronic nicotine exposure, suggesting a potential therapeutic effect of nicotine on CCI-induced motor dysfunction. Previous studies have shown that the activation of α7 nAchR can contribute to increased neuronal survival in the hippocampus after brain injury [44,45]. Corresponding to this previous finding, we observed increased fl-TrkB receptor expression in CCI mice that received chronic nicotine exposure, suggesting that the activation of nAchRs may enhance BDNF-TrkB signaling, which translates to increased neuronal survival and recovery [46]. Additionally, an attenuation of excessive inflammatory response was previously reported in rat ischemic and stroke models after α7 nAchRs activation [45]. Thus, it is plausible that the improved nestling outcome may be interpreted as a consequence of neuroprotective effects associated with nAchRs activation.

We have previously demonstrated that our CCI model disrupted hippocampus-dependent contextual memory recall but not fear memory acquisition and cue-dependent memory retrieval [2]. In this current study, no deficit was observed in contextual and cue-dependent memory recall

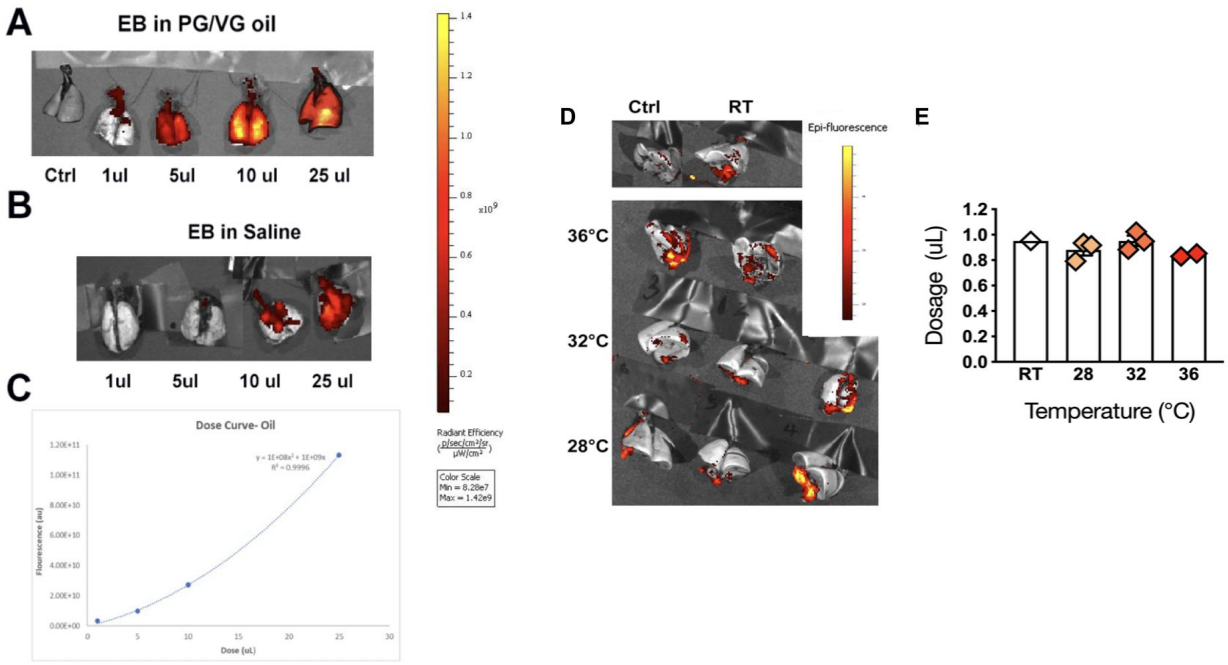
among all four groups (**Supplemental Figure 2**). We suspect environmental stressors from six weeks of vapor exposure may have interfered with fear memory acquisition and retrieval. The similarity between the textures of the grid/wire flooring in the exposure chamber and the fear conditioning chamber may have also decreased the sensitivity of these memory retrieval tests.

In summary, CCI and chronic nicotine exposure inhibited social motivation and impaired novel object recognition memory. The observed behavioral deficits, in part, may be induced by the loss of hippocampal β2-containing nAchRs due to CCI and chronic nicotine vaping. In this current study, we did not observe a significant change in hippocampal α7 nAchRs, another functionally important receptor that responds to nicotine. Sparks (1999), Besson (2007), and colleagues have previously reported that chronic nicotine exposure altered α7 nAchRs expression only in a few brain regions [28,47]; thus, there may be unobserved biochemical changes beyond the hippocampus. To more accurately characterize the compounding effects of chronic nicotine exposure and CCI on the CNS, circuit-level analysis of cholinergic and dopaminergic neural networks may be required. Additionally, we will next perform immunohistochemistry to evaluate the effects of CCI and chronic nicotine exposure on micro- and astrogliosis, which predict long-term outcomes in TBI pathology. Importantly, a few limitations of our current experimental protocols warrant further discussion and examination. Previous studies have reported that both acute and prolonged abstinence from nicotine could alter the nAchRs expression in the brain [48,49]. In this current study, since behavioral tests and tissue collection were completed three weeks after the cessation of nicotine exposure; therefore, it is unclear if nicotine-induced behavioral and biochemical changes were captured in a timely manner. For the following studies, separate cohorts of mice may be required to assess behavioral and biochemical changes

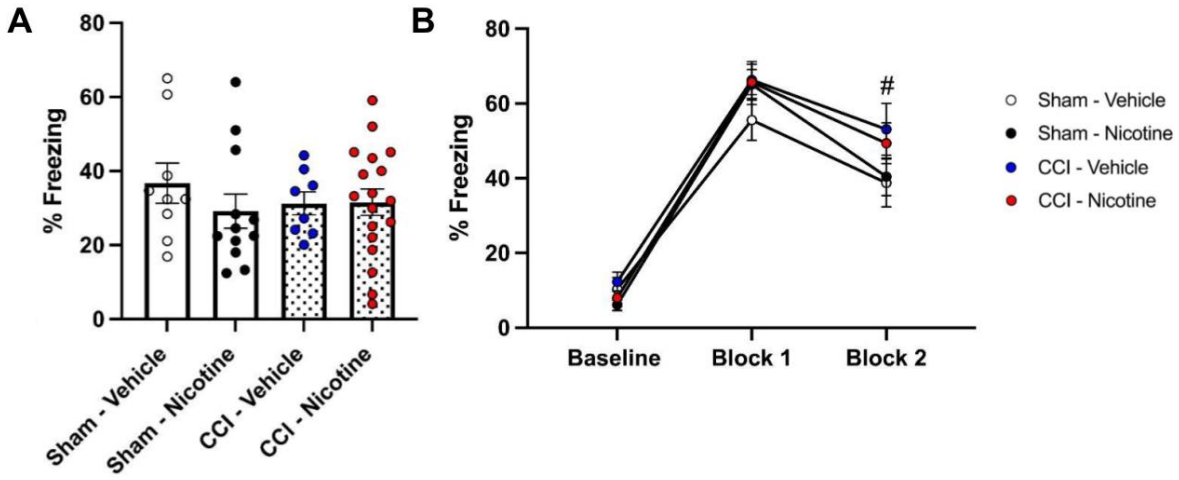
immediately after the last vaping exposure session. Additionally, further optimization of the vapor exposure chamber is necessary to minimize environmental stressors, including noise from the vacuum pump, vapor puffs, and vibrations from the fume hood.

As E-cigarette is gaining popularity among adults and teenagers, it is critical to understand the effects of chronic vaping to prevent unexpected risk factors and nicotine abuse. The current study provides a detailed analysis of neurobehavioral changes in CCI mice with chronic nicotine exposure. However, the precise biochemical and electrophysiological mechanisms underlying these observed behavioral changes remain unexplored. Thus, an urgent collaboration among neurobiologists, chemists, and clinicians is needed to understand the long-term impacts of E-cig smoking on public health.

The current thesis contains materials being prepared for publication. Mr. Wang was the principal author of this thesis.



Supplemental Figure 1. Determination of optimal chamber exposure temperature. (**A-B**) Images of fluorescent lungs generated via direct tracheal injection of Evans Blue (EB) dye dissolved in vehicle and saline solutions. (**C**) A standard curve for dosage vs. fluorescence intensity. (**D**) Fluorescent lungs from temperature-dependent exposure studies. (**E**) Quantification of D reveals no difference in EB concentration by varying exposure chamber temperature.



Supplemental Figure 2. Contextual and cue-dependent memory recall are unaffected by CCI and chronic nicotine exposure. (**A**) No change in percent freezing observed during assessment of contextual memory recall. (**B**) No difference in percent freezing was observed during block 1 test for cue-dependent memory recall. The CCI-Vehicle group exhibited increased percent freezing compared to the Sham-Vehicle group during block 2 test for fear memory extinguishment. Fear conditioning data were analyzed using two-way ANOVA by Fisher's LSD tests. Significance was assumed when $p < 0.05$. #$p < 0.05$.

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