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Nicotine Administration Normalizes Behavioral and Neurophysiological Perturbations in the MAM Rodent Model of Schizophrenia

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Abstract

Background: The present study utilized the methylazoxymethanol (MAM) neurodevelopmental rodent model of schizophrenia (SCZ) to evaluate the hypothesis that individuals with SCZ smoke in an attempt to "self-medicate" their symptoms through nicotine (NIC) intake.
Methods: To explore this question, we examined the effects of acute and chronic administration of NIC in 2 established behavioral tests known to be disrupted in the MAM model: prepulse inhibition of startle and novel object recognition. Additionally, we assessed the effects of acute and chronic NIC on 2 indices of the pathophysiology of SCZ modeled by MAM, elevated dopamine neuron population activity in the ventral tegmental area and neuronal activity in the ventral hippocampus, using in vivo electrophysiological recordings.
Results: Our findings demonstrated that both acute and chronic administration of NIC significantly improved deficits in prepulse inhibition of startle and novel object recognition among MAM rats and normalized elevated ventral tegmental area and ventral hippocampal neuronal activity in these animals.
Conclusion: Together, these findings of NIC-induced improvement of deficits lend support for a "self-medication" hypothesis behind increased cigarette smoking in SCZ and illustrate the potential utility of nicotinic modulation in future pharmacotherapies for certain SCZ symptoms.

Keywords: Dopamine, nicotine, schizophrenia, self-medication

Introduction

An estimated 60% of individuals with schizophrenia (SCZ) are current cigarette smokers, representing roughly 3 times the proportion of smokers in the general population (Dickerson et al., 2018). The elevated rate of smoking among SCZ patients is hypothesized to be an attempt to "self-medicate" negative and cognitive symptoms of this disorder through intake of nicotine (NIC), the primary reinforcing component of tobacco (Kumari and Postma, 2005). Although direct experimental tests of whether therapeutic effects of NIC on certain SCZ symptoms is a motivator of smoking have yielded mixed results, evidence does suggest consistent modulatory effects of nicotinic agonists on particular deficits in SCZ (AhnAllen, 2012; Tregellas and Wylie, 2019). A better understanding of the factors driving smoking and the neurophysiological effects of NIC that may be unique to SCZ smokers is critical to reducing tobacco use in this population and could provide important insight into novel approaches to managing symptoms of SCZ.

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Significance Statement

The rate of smoking among those with schizophrenia is approximately 3 times higher than that of the general population, contributing to disparities in health and social well-being. This heavy smoking is hypothesized to be an attempt to "self-medicate" symptoms of schizophrenia, though direct experimental tests of this hypothesis in humans are challenging and have produced mixed results. However, evidence suggests consistent effects of nicotine on a number of deficits observed in schizophrenia, which are also observed in animal models of the disorder. We utilized the methylazoxymethanol acetate (MAM) rodent model of schizophrenia to explore the effects of nicotine administration on several behavioral and physiological disruptions observed in the model. Our findings demonstrated that both acute and chronic administration of nicotine significantly improved both sensory and cognitive deficits among MAM rats and normalized elevated neuronal activity in these animals. Together, these findings lend support for a "self-medication" hypothesis behind increased smoking in schizophrenia and illustrate the potential utility of nicotinic receptor modulation in future pharmacotherapies for schizophrenia symptoms.

The majority of studies testing the hypothesis that smoking serves as a form of "self-medication" in SCZ have focused on measurable behavioral effects of NIC. Although findings are mixed, the weight of evidence suggests that NIC can acutely improve negative symptom severity and some measures of cognitive function, particularly attention, in patients (Smith et al., 2002; Harris et al., 2004; Postma et al., 2006; Dondé et al., 2020). Comparison of cognitive symptom severity in SCZ smokers vs nonsmokers may not be an ideal metric to inform the self-medication hypothesis, as one cannot determine whether being a smoker impacts cognitive status, or if those with more severe symptoms are more likely to smoke (Taiminen et al., 1998; Wing et al., 2011). Because of this, experimental assessments of NIC effects on cognition in SCZ smokers and nonsmokers are essential. Acute transdermal NIC administration can improve attentional performance, response inhibition, and episodic memory in SCZ nonsmokers (Barr et al., 2008; Jubelt et al., 2008). Cigarette smoking has also been demonstrated to improve visuospatial working memory performance in SCZ smokers, while acute smoking abstinence selectively exacerbates impairments in this group (George et al., 2002; Sacco et al., 2005). NIC administration also consistently improves deficits in sensory gating, a key neurological process that functions to filter redundant or unnecessary stimuli from higher cortical processing, preventing an excess of less relevant environmental stimuli from interfering with processing of more salient events. Deficits in sensory gating measured by tactile or auditory prepulse inhibition of startle (PPI) in response to successive stimuli are a reliable clinical marker of SCZ. NIC administration consistently enhances auditory and tactile PPI in both control and SCZ smokers and nonsmokers (Dondé et al., 2020), suggesting a direct pharmacological effect of NIC.

While SCZ is a distinctly human disorder with no precisely determined etiology, animal models capturing known neurobiological disruptions observed in the affected brain are a versatile tool in mechanistic studies. Models developed through pharmacological manipulation or neurodevelopmental insult, for example, display systems-level neuronal dysfunctions that parallel many observations in SCZ. The methylazoxymethanol acetate (MAM) model is a well-validated neurodevelopmental disruption model of SCZ highlighted by temporal lobe dysfunction and resulting uncoordinated activity of ventral hippocampus (vHipp) and its downstream projection regions, including ventral tegmental area (VTA) and medial PFC (Esmaeili and Grace, 2013; Modinos et al., 2015). One study demonstrated that $\alpha 7$ nAChR agonist administration, either systemically or directly into vHipp, normalized elevated dopamine (DA) population activity observed in MAM rats but had no effect on controls (Neves and Grace, 2018). This finding has potential implications for nicotinic effects on psychosis, which is largely thought to be a result of DA hyperactivity in SCZ, as well as greater system-wide effects.

The present studies aim to evaluate the effects of acute and chronic NIC treatment on behavioral and neurophysiological disruptions observed in the MAM rodent model of SCZ. To assess effects of NIC on sensory gating and episodic memory deficits in MAM rats, we measured auditory PPI and novel object recognition (NOR), respectively, in MAM and CTL rats before and after NIC administration. To determine whether NIC administration could normalize elevated VTA and vHipp neuronal activity in MAM rats, we also performed electrophysiological recordings before and after NIC administration. We hypothesized that acute administration of NIC, both in NIC-naïve and chronically treated animals, would improve deficiencies in these measures in MAM rats.

METHODS

Subjects

All experiments utilized Sprague-Dawley rats aged 2 to 6 months at the start of experimental sessions; although a range of ages were used in individual experiments, rat ages were matched across all treatment groups within an experiment. Rats were born in-house to timed pregnant dams (Envigo, Indianapolis, IN, USA) injected i.p. with saline (CTL; 1.0 mL/kg) or MAM MRI Global, Kansas City, MO, USA; 25.0 mg/kg, 1.0 mL/kg in saline) on GD17 (Lodge, 2013). After weaning on postnatal day 22 (P22), pups were separated by treatment group and sex and housed in groups of 2 to 3 in tub cages with woodchip bedding. At P60 to 70 (prior to the start of experiments), animals were housed individually. Each experiment utilized both male and female animals from 2 to 3 MAM-treated and 2 to 3 CTL litters. Animals had ad libitum access to food and water in the home cage throughout the duration of the experiment. Facilities were maintained on a reversed 12-hour-light/-dark cycle (lights off 7:00 AM), with experiments performed during the dark phase. Behavioral sessions and electrophysiological recordings were performed under dim light. All experiments were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

NIC Treatment

NIC hydrogen tartrate salt (MP Pharmaceuticals, Solon, OH, USA) was dissolved in 0.9% sterile saline (SAL), passed through a 0.22-μM filter to ensure sterility, and stored for up to 4 weeks at 4°C with minimal light exposure until use. Doses are expressed as

free base. Animals in chronic NIC experiments were injected with NIC (0.3 mg/kg, s.c.) daily for 12 to 24 days prior to behavioral testing or recordings, with the duration of treatment between MAM and CTL rats being matched within experiments. A moderate dose of NIC (0.3 mg/kg, s.c.) was selected to minimize adverse effects on behavior, such as lethargy, while maintaining relevance to dosing in smokers (Matta et al., 2007). Those tested in acute NIC experiments received injections of SAL (1.0 mL/kg, s.c.) daily for at least 12 days to control for effects of repeated injections. Acute NIC infusions (30 μg/kg, i.v.) during electrophysiological recording sessions were delivered through a catheter inserted into the right jugular vein.

Prepulse Inhibition of Startle

Assessment of auditory PPI in MAM and CTL animals was adapted from Moore et al. (2006). Thirty minutes after injection with NIC or SAL, rats were placed into transparent Plexiglas restraint tubes inside startle chambers (San Diego Instruments, Inc., San Diego, CA) and habituated to a 60-dB white noise background for 5 minutes. PPI was determined using 21 trials of a 105-dB startle tone (40 milliseconds in duration) preceded by prepulses 7 or 11 dB above background (12 milliseconds in duration) with an intertrial interval of 15 to 25 seconds. Seven trials contained the startle tone alone. Startle response (measured as arbitrary force units) was measured for each trial, and percent PPI was calculated for each prepulse intensity as a percent change in response compared with startle-only trials. Animals in both groups were tested once after SAL injection (pre-NIC baseline; 24 hours abstinent for chronic NIC animals) and again after NIC injection (post-NIC). These trials were separated by at least 48 hours.

Novel Object Recognition

Behavioral testing took place in a black Plexiglas arena (43 cm W × 43 cm L × 32 cm H) in a dimly lit testing room as described by Stark et al. (2019). For each session, animals were placed in the arena with 2 identical objects placed in opposite corners and allowed to explore for 5 minutes (familiarization phase), after which they were returned to their transport cage for an inter-trial interval of 1 hour. Objects used were brown glass bottles, metallic cylinders, and nonporous ceramic figurines 10 to 15 cm in height, and pairings of novel and familiar objects were randomized across animals. Fifteen minutes before the testing phase, animals were injected with NIC (0.3 mg/kg, s.c.) or SAL (1.0 mL/kg). During the testing phase, animals were again placed in the Plexiglas arena with 1 familiar object and 1 novel object (placement in corners was counterbalanced across animals) and again allowed to explore freely for 5 minutes. This exploration was recorded, and videos were later scored for interaction time with each object by an experimenter blinded to subjects' drug conditions, with percent interaction time with novel object measured ([novel time/(total interaction time)] × 100). "Interaction" was defined as touching, sniffing, or orientation of the snout toward the object within a distance of <2 cm. Sitting on top of the object was not scored as interaction. The arena and all objects were cleaned with 70% ethanol between each testing period.

Electrophysiological Recordings

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p., supplemented as necessary; Sigma-Aldrich, St. Louis, MO, USA), and an infusion catheter was surgically placed in the right jugular vein. Rats were then secured into a stereotaxic apparatus (Kopf, Tujunga, CA, USA), and a core body temperature of 37°C was maintained using a thermostatically regulated heating pad. The skull was then surgically exposed and the recording site prepared. VTA DA neuron activity was measured using extracellular recordings as described previously (Neves and Grace, 2018). Single glass microelectrodes filled with 2% Chicago Sky Blue dye in 2M NaCl were lowered into the ventral tegmental area (AP + 5.3, ML − 0.6 relative to bregma) using a hydraulic micropositioner. Spontaneously active neurons were sampled between −6.5 and −9.0 mm DV using a 30-Hz highpass and 16-kHz lowpass filters. Putative DA neurons meeting electrophysiological criteria of location, waveform, and firing rate/pattern were isolated and recorded for at least 60 seconds (Ungless and Grace, 2012). Baseline population activity was determined by making 3 to 4 vertical passes through the VTA in a random arrangement moving laterally and caudally, separated by 200 μm, and counting the number of active DA neurons per track as well as their firing rate and burst pattern. Animals were then infused with NIC (30 μg/kg, i.v.), and 3 to 4 more tracks were recorded to determine effects of systemic NIC on DA neuron population activity as well as individual cells' firing rates (Hz) and percentage of spikes in bursts.

Extracellular recordings in vHipp were performed as described previously (Lodge and Grace, 2007). Single glass microelectrodes filled with 2% Chicago Sky Blue dye in 2M NaCl were lowered into the ventral hippocampus (AP + 6.0, ML − 4.5 relative to bregma) to a depth of −6.0 to −8.0 mm ventral to the brain surface. Spontaneously active neurons throughout the region were recorded for at least 120 seconds each in a series of tracks moving laterally and caudally from the first, separated by 200 μm. As described for DA neuron recordings, 2 to 4 vertical passes were made for baseline recordings and 2 to 4 more were made following acute infusion of NIC. Putative pyramidal cells were identified as those with spikes in a biphasic waveform, a spike duration >2.0 ms, and a firing rate <2 Hz.

Histology

At the conclusion of each electrophysiological recording, rats were killed with an overdose of chloral hydrate (additional 400 mg/kg, i.p.). The recording site was marked by electrophoretic injection of Chicago Sky blue dye (−20 μA for 20–30 minutes). Animals were then decapitated and brains removed and fixed in 8% paraformaldehyde in 0.2 M phosphate buffered saline and subsequently cryoprotected in 25% sucrose in 0.2 M phosphate buffered saline. Sixty-micrometer sections were taken throughout VTA or vHipp and stained with 95% Neutral Red and 5% Cresyl Violet to verify electrode placement and MAM phenotype. Due to laboratory closures caused by the COVID-19 pandemic, brains from a subset of animals in the Acute NIC vHipp recording experiment could not be processed.

Data Analysis

Data analyses were conducted using IBM SPSS Statistics (Armonk, NY, USA) and GraphPad Prism (GraphPad Software, San Diego, CA, USA). Data from PPI and NOR experiments were analyzed using separate 2-way repeated-measures ANOVA for acute vs chronic NIC animals, with group (MAM vs CTL) and drug (pre- vs post-NIC) as factors. A range of rat ages were used in individual experiments with ages of rats in MAM and CTL groups being matched within experiments, but no age-related differences or trends were noted in the data. Also, rats treated with chronic NIC were treated for 12

to 24 days (matched across treatment groups), but no differences or trends based on duration of treatment were noted in the data. Because no sex differences in key parameters were anticipated or observed in the data collected, data from male and female animals were pooled for analyses. However, 1 sex difference was noted: in the PPI test, baseline startle force was significantly lower in females compared with males (F1,22 = 10.11, $P < .005$), reflecting the lower body weight of the female rats. Even so, the key measured parameter in this test, % PPI, did not differ between males and females. Data collected during electrophysiological recordings were analyzed using LabChart and NeuroExplorer software to determine firing rate and, in DA neuron recordings, percentage of spikes occurring in bursts (Grace and Bunney, 1983; Neves and Grace, 2018). DA population activity, defined as the average number of spontaneously active neurons encountered per electrode track, was calculated for each animal at baseline and after NIC infusion. Values are reported as mean ± SEM unless otherwise stated. Analyses of firing rate, bursting, and population activity within each experiment were conducted using a 2-way mixed ANOVA, with group (MAM vs CTL) as between-subjects factors and drug (baseline vs post-NIC) as within-subjects factors when possible, followed by post-hoc *t* tests ($\alpha = .05$; reported *P* values are adjusted for multiple comparisons using Bonferroni correction).

RESULTS

PPI Deficits in MAM Rats Are Mitigated by Acute and Chronic NIC Administration

Percent inhibition of the startle response by 7-dB and 11-dB prepulses was measured in MAM (n = 13, 4 M/9 F) and CTL (n = 13, 7 M/6 F) rats. Although MAM rats demonstrated a PPI deficit at both 7-dB and 11-dB prepulse intensities, only the analyses from 7-dB trial data are summarized here due to high variability in MAM rats' responses at 11 dB. Although baseline startle response was lower in the MAM group compared with the CTL group (F1,22 = 4.88, $P < .05$), likely because of the greater representation of females in this group, which have lighter body weights and therefore smaller startle forces, % PPI did not vary as a function of baseline startle response. Also, NIC treatment did not significantly alter baseline startle. In the acute NIC experiment, there was an effect of group ($F_{1,24} = 4.69$, $P < .05$) and of drug ($F_{1,23} = 18.52$, $P < .0005$) and a group × drug interaction ($F_{1,23} = 4.68$, $P < .05$) on % PPI (Figure 1A). Post-hoc *t* tests confirmed that, at pre-NIC baseline, MAM rats demonstrated a significantly lower % PPI than CTL animals (–2.52% ± 9.24% vs 27.75% ± 7.18%; $P < .05$). Acute NIC injection significantly increased % PPI in MAM rats ($t_{12} = 4.17$, $P < .005$). In the chronic NIC experiment, there were no significant drug or group or a drug × group interactions (Figure 1B). MAM (n = 12, 5 M/7 F) and CTL (n = 7, 4 M/3 F) animals did not differ in % PPI at pre-NIC baseline (24 hours abstinent from NIC) (26.60% ± 8.82% vs 39.58% ± 8.89%; $P = .35$). NIC administration further increased % PPI from pre-NIC baseline in MAM rats (46.20% ± 5.67%; $P < .05$).

Acute and Chronic NIC Normalizes NOR in MAM Rats

Total object interaction times in the acute and chronic NIC administration in NOR experiments were compared for MAM and CTL animals to confirm comparable exploration between groups. There were no significant effects of group ($P = .93$) or drug ($P = .36$) and no group × drug interaction ($P = .66$) on total object interaction time in the acute NIC experiment. In the chronic NIC experiment, there was no effect of group ($P = .56$) and no group × drug interaction ($P = .76$), but there was a significant effect of drug ($F_{1,12} = 13.51$, $P < .005$). Total object interaction time was significantly reduced in both groups after NIC administration, but they did not differ at either timepoint. A 2-way repeated-measures ANOVA of percent interaction time with novel object in drug-naïve MAM (n = 9, 4 M/5 F) and CTL animals (n = 8, 4 M/4 F) before and after acute NIC revealed a group × drug interaction ($F_{1,15} = 12.05$, $P < .005$; Figure 2A). At pre-NIC baseline, MAM rats spent a significantly lower percentage of time interacting with the novel object than CTL animals (62.70% ± 4.03% vs 75.02% ± 1.82%; $P < .05$). However, MAM rats spent a significantly greater percentage of time interacting with the novel object after NIC administration compared with pre-NIC baseline (74.07% ± 2.46%; $P < .05$) and did not differ from CTL animals post-NIC (68.41% ± 4.04%; $P = .24$). In the chronic NIC experiment, there was no group × drug interaction and no effect of group or drug on percent interaction time with novel object (Figure 2B). MAM (n = 7, 3 M/4 F) and CTL (n = 7, 4 M/3 F) animals did not differ when tested after 24 hours abstinent from NIC (pre-NIC baseline; $P = .19$) or after NIC injection (post-NIC; $P = .65$).

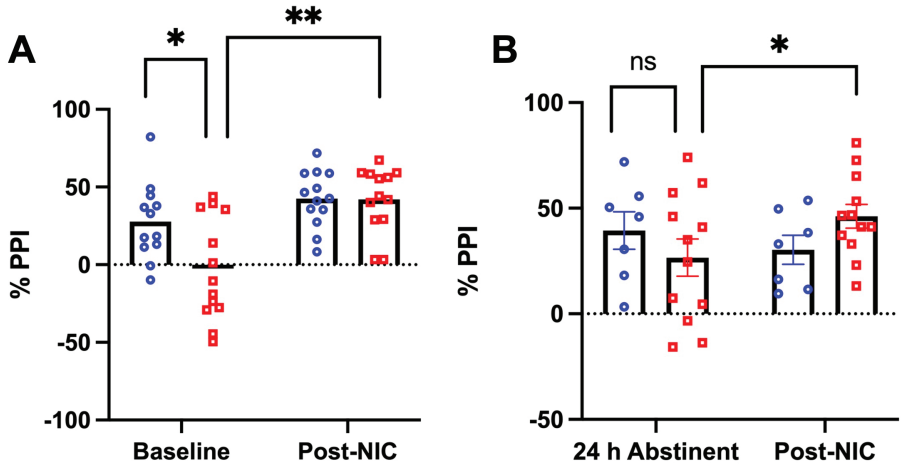

Figure 1. Acute and chronic NIC normalizes prepulse inhibition of startle in MAM animals. Average percent inhibition of startle response in prepulse trials (% PPI). (A) CTL animals demonstrated significantly higher % PPI at Baseline than MAM animals. Acute NIC normalized this difference, significantly increasing % PPI in MAM subjects. (B) Chronically NIC-treated CTL and MAM animals did not differ in % PPI at a 24-hour abstinent baseline, and NIC injection further increased % PPI in MAM animals. CTL rats, blue circles; MAM rats, red squares; * $P < .05$; ** $P < .01$. Abbreviations: CTL, control; MAM, methylazoxymethanol acetate; NIC, nicotine; PPI, prepulse inhibition.

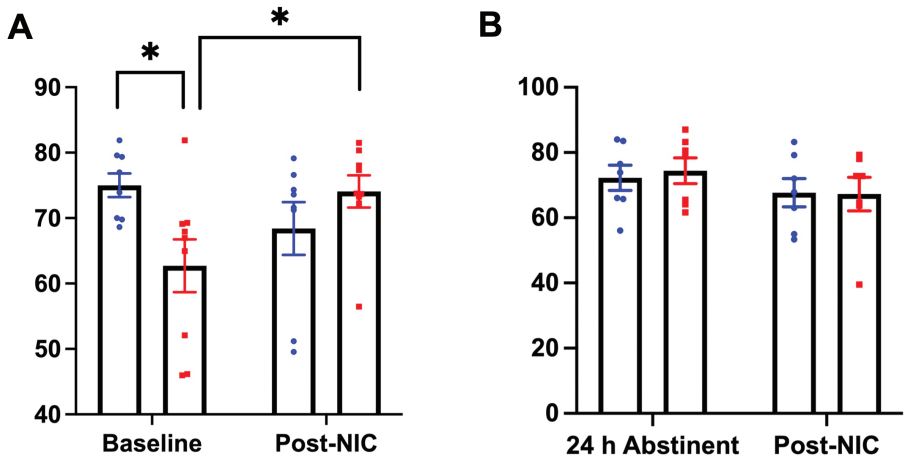

Figure 2. Acute and chronic NIC normalizes novel object recognition in MAM animals. Percent total interaction time spent with novel object during NOR task. (A) At baseline, CTL animals spent a significantly greater proportion of time interacting with the novel object than MAM animals. Acute NIC administration significantly increased MAM animals' interaction time with the novel object to a level comparable with CTL animals. (B) Chronically NIC-treated MAM and CTL animals did not differ in percent interaction time with the novel object at a 24-hour abstinent baseline or after acute NIC administration. CTL rats, blue circles; MAM rats, red squares; * $P < .05$. Abbreviations: CTL, control; MAM, methylazoxymethanol acetate; NOR, novel object recognition; NIC, nicotine.

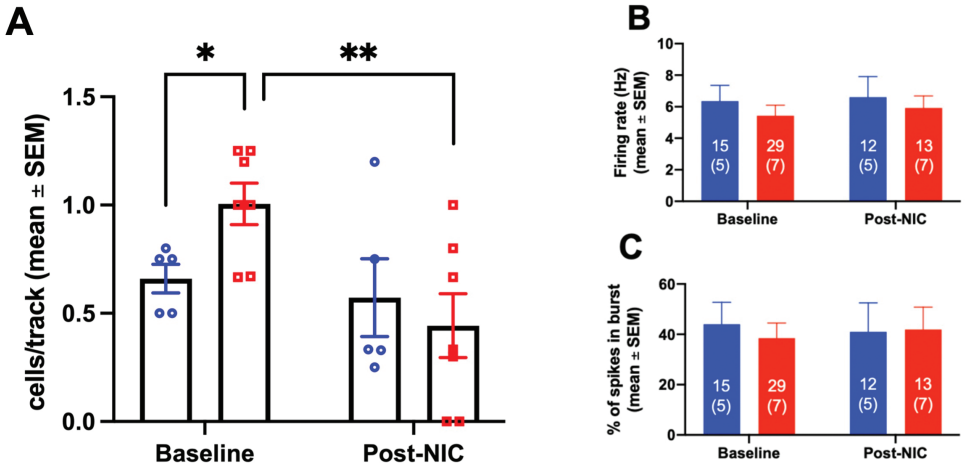

Figure 3. Acute NIC normalizes elevated DA population activity in MAM animals. Electrophysiological data recorded from spontaneously active neurons in VTA. (A) At baseline, population activity of VTA DA neurons was significantly higher in MAM than CTL animals. Acute systemic NIC infusion normalized this elevated activity, with the number of DA neurons encountered in MAM rats significantly less relative to baseline. (B, C) MAM and CTL animals did not differ in mean firing rate or percentage of spikes in burst at baseline or post-NIC. CTL rats, blue circles/bars; MAM rats, red squares/bars; * $P < .05$; ** $P < .01$; bar labels indicate number of cells analyzed (number of animals). Abbreviations: CTL, control; DA, dopamine; MAM, methylazoxymethanol acetate; NIC, nicotine; VTA - ventral tegmental area.

Acute Treatment Normalizes DA Population Activity in MAM Rats

Analysis of population activity in drug-naïve MAM (n = 7 rats; 3 M/4 F) and CTL animals (n = 5 rats; 4 M/1 F) at baseline and after NIC treatment (acute NIC) revealed a significant effect of drug ($F_{1,10} = 9.0$; $P < .05$) and a significant group × drug interaction ($F_{1,10} = 4.8$; $P = .052$). Post-hoc tests indicated that MAM rats showed significantly elevated DA population activity at baseline (1.01 ± 0.01 cells/track) relative to CTL animals (0.66 ± 0.07 cells/track; $P < .05$). However, as hypothesized, population activity in MAM rats was significantly reduced after NIC administration (0.44 ± 0.15 cells/track; $P < .01$; Figure 3A). There were no significant effects by group or drug on average firing rate or on bursting (Figure 3B–C).

For assessment of chronic NIC effects, MAM and CTL rats (n = 6 per group; 4 M/2 F) were injected with NIC (0.3 mg/kg, s.c.) daily for at least 12 days. When measured 24 hours after their last injection (baseline) and then after a single infusion of NIC (post-NIC), there was no effect by drug ($P = .18$) or group ($P = .29$) and no drug × group interaction ($P = .56$) on VTA DA population activity in MAM and CTL rats (Figure 4A). Similarly, there were no effects on average firing rate or on bursting in these animals (Figure 4B–C).

Acute and Chronic NIC Normalizes Elevated vHipp Firing Rates in MAM Rats

Analysis of average firing rates of putative pyramidal neurons in vHipp of MAM (n = 8 rats; 5 M/3 F) and CTL (n = 8 rats; 5 M/3 F) rats revealed a significant effect of group ($F_{1,28} = 5.96$, $P < .05$, Figure 5A) but no effect of drug ($P = .27$) and no group × drug interaction ($P = .17$). Student's *t* test of average firing rates in drug-naïve animals was consistent with our prior reports, showing that firing rates in MAM rats (1.23 ± 0.1 Hz) were significantly higher than those in CTL animals (0.84 ± 0.11 Hz) measured at baseline ($t_{14} = 2.69$, $P < .05$) (Lodge and Grace, 2007). Additionally, average post-NIC firing rates (0.98 ± 0.11 Hz) in MAM rats were significantly

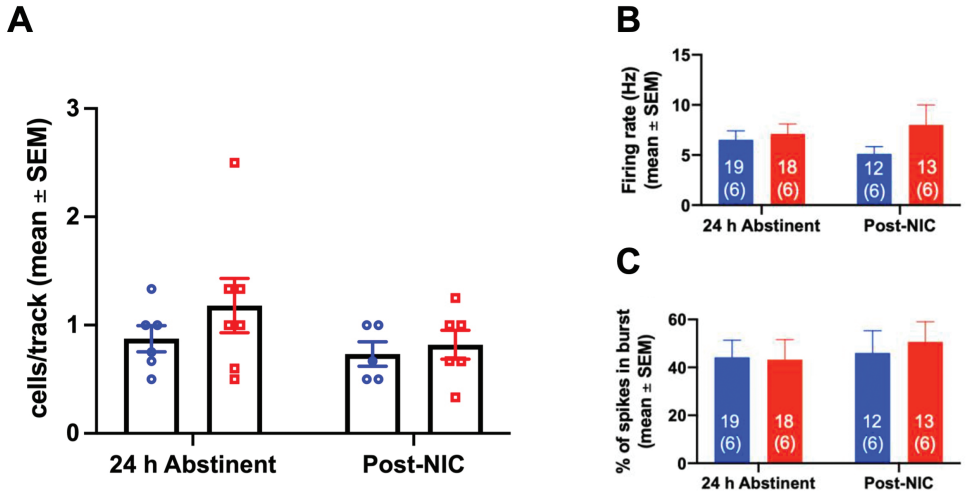

Figure 4. VTA DA neuron activity is comparable in CTL and MAM animals after chronic NIC. Electrophysiological data collected from spontaneously active neurons in VTA. (A) CTL and MAM animals chronically treated with NIC demonstrated comparable VTA DA population activity at a 24-hours abstinent baseline and after acute NIC infusion. (B, C) MAM and CTL animals did not differ in mean firing rate or percentage of spikes in burst at baseline or post-NIC. CTL rats, blue circles/bars; MAM rats, red squares/bars; bar labels indicate number of cells analyzed (number of animals). Abbreviations: CTL, control; DA, dopamine; MAM, methylazoxymethanol acetate; NIC, nicotine; VTA - ventral tegmental area.

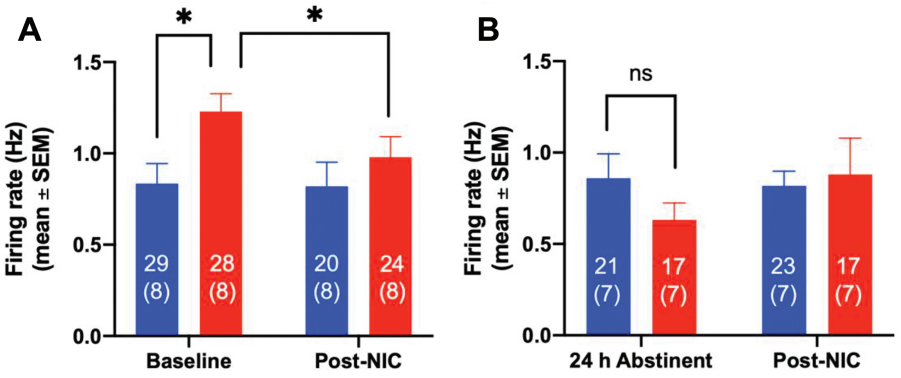

Figure 5. Acute and chronic NIC normalizes elevated firing rates of vHipp neurons. (A) Mean firing rates of spontaneously active neurons encountered in vHipp were significantly higher in NIC-naïve MAM animals than CTL at baseline. However, acute systemic infusion of NIC vHipp neuronal firing rates were significantly lower in MAM animals, normalizing this elevation. (B) vHipp neuronal firing rates measured in chronically NIC-treated MAM and CTL animals did not differ when measured at a 24-hours abstinent baseline or after acute NIC infusion. CTL rats, blue bars; MAM rats, red bars; *$P<.05$; bar labels indicate number of cells analyzed (number of animals). Abbreviations: CTL, control; MAM, methylazoxymethanol acetate; NIC, nicotine; vHipp, ventral hippocampus.

lower than those measured at baseline ($t_7=2.45$, $P<.05$) and not significantly different from baseline firing rates in CTL animals ($P=.38$). For assessment of chronic NIC effects, as in the DA recording experiment, MAM (n=7 rats; 4 M/3 F) and CTL rats (n=7 rats; 3 M/4 F) were injected with NIC (0.3 mg/kg, s.c.) daily for at least 12 days. A 2-way ANOVA of average neuronal firing rates in each animal revealed no significant effects by group ($P=.53$) or drug ($P=.43$) and no group × drug interaction ($P=0.27$; Figure 5B). As hypothesized, mean firing rates of cells recorded from vHipp in chronically treated MAM (0.88 ± 0.2 Hz; n=17 cells/7 animals) and CTL animals (0.82 ± 0.08 Hz; n=23 cells/7 animals) did not differ after acute NIC infusion ($t_{11}=0.31$, $P=.76$).

DISCUSSION

Our work aims to explore mechanisms underlying increased smoking in SCZ using the MAM rodent model of the disorder. Previously, we demonstrated that NIC reinforcement is not increased in these animals, failing to support the hypothesis that SCZ pathophysiology confers increased NIC reward driving continued NIC use (Weeks et al., 2020). We therefore aimed to evaluate the self-medication hypothesis, which proposes that NIC normalizes some cognitive symptoms of SCZ, in this study. These experiments sought to characterize the effects of NIC treatment on measures of sensory gating and cognitive deficits, as well as underlying neurophysiological perturbations, in this well-established rodent model of SCZ. Our findings suggest that both acute and chronic administration of NIC can normalize sensorimotor gating, short-term memory deficits, and elevated VTA and vHipp neuronal activity in the MAM rodent model of SCZ. To our knowledge, this study is the first to examine the effects of NIC on these measures in the MAM model and is unique among studies in animal models of SCZ in its comparison of the effects of single and multiple NIC administrations.

Our study utilized auditory PPI as a measure of sensorimotor gating in MAM and CTL rats. This assessment is a consistently demonstrated analog of sensorimotor gating dysfunction in SCZ reproduced reliably by the MAM model and that is known to be modulated by nicotinic receptors (Swerdlow et al., 1999; Ewing and Grace, 2013; Pinnock et al., 2015). Our experiments confirmed

a reduction in baseline PPI in MAM rats relative to CTL, which was significantly increased and fully normalized by an acute injection of NIC. This replicates clinical findings of NIC enhancement of PPI in CTL nonsmokers and parallels NIC normalization of P50 sensory gating deficits in nonsmoking first-degree SCZ relatives (Adler et al., 1993; Kumari et al., 1997). Our experiments also sought to assess the effects of NIC on episodic memory and novelty discrimination in the MAM model using the NOR task, a metric in which MAM rats consistently display deficits (Flagstad et al., 2005; Stark et al., 2019). We demonstrated that acute NIC injection normalizes the significant deficit in NOR performance observed in NIC-naive MAM rats. While chronically treated MAM and CTL rats did not differ in NOR after acute NIC injection, acute NIC further enhanced PPI in chronically treated MAM rats, suggesting that these animals did not develop tolerance to the PPI-enhancing effects of NIC. However, the use of a single, experimenter-administered daily dose in our chronic treatment protocol does not necessarily reflect human smoking, as smokers are able to self-regulate NIC intake by choosing when and how many cigarettes to smoke. Though MAM and CTL animals do not differ in NIC self-administration and thus experience similar reinforcing effects of NIC, it is possible that the cognitive effects we observed may differ across different dosing paradigms (Weeks et al., 2020). Nonetheless, our findings do suggest that acute NIC normalizes PPI and NOR deficits in MAM rats and that this effect persists even after chronic NIC administration. Importantly, these findings also validate the MAM model as a useful tool in future studies of the effects of NIC and nicotinic drugs on sensory gating deficits and memory dysfunction in SCZ.

To date, no published study, to our knowledge, has examined the effects of NIC on neurophysiological dysfunction observed in the MAM model. However, Neves et al. (2018) found that α7 nAChR agonist administration normalized elevated VTA DA population activity in MAM rats via an action in vHipp, which led us to hypothesize that administration of NIC may produce similar results. Co-labeling experiments have indicated that both α4β2 and α7 nAChRs are highly expressed on parvalbumin-positive inhibitory interneurons in the hippocampus, and decreased expression of parvalbumin-positive and functional α7 nAChRs are well-established findings in studies of post-mortem tissue in SCZ (Freedman et al., 1995, 2000; Leonard et al., 2002). Together, these findings suggest a mechanism by which activation of both high-affinity α4β2 and low-affinity α7 nAChRs by high NIC concentrations may serve to potentiate activation of these regions of reduced expression and thus mitigate imbalances in excitation and inhibition that contribute to the MAM endophenotype. Here, we propose that activation of nAChRs on inhibitory interneurons of vHipp may serve to normalize increased activity of vHipp pyramidal cells and, in turn, restore proper inhibition of the NAcc-ventral pallidum GABAergic input, which normalizes the increased DA population activity observed in VTA. While we did find that both acute and chronic systemic NIC normalized hyperactivity in both vHipp and VTA in MAM rats as hypothesized, our results cannot confirm that these effects occur via direct action of NIC on nAChRs in vHipp and its resulting drive to VTA inputs, nor can we directly connect this effect with our behavioral findings. The use of systemic rather than locally administered NIC in these experiments was justified by its relevance to NIC intake in human smokers. However, these results are consistent with the findings of Bortz and Grace (2018), which demonstrated that cholinergic activation of the ventral subiculum of Hipp is a key moderator of increases in VTA DA neuron population activity, lending support to the proposed pathway of action. Results of prior clinical and preclinical studies illustrating the particular role of vHipp α7 nAChRs in SCZ-related neuronal dysfunction converge with our finding that the effects of NIC on VTA and vHipp neuronal activity were specific to MAM rats and not observed in CTL rats (Freedman et al., 2000; Kohlhaas et al., 2015; Neves and Grace, 2018). Additionally, the observed lack of an effect of NIC at the dose tested on firing rates of individual DA neurons in anesthetized animals was consistent with prior literature (Grenhoff et al., 1986; Mereu et al., 1987). Though not examined behaviorally in the MAM model, α7 nAChR agonist administration was found to improve deficits in NOR and normalized decreases in parvalbumin and α7 nAChR expression produced by subchronic MK-801 exposure, another preclinical model of SCZ-like symptoms (Unal et al., 2021). However, clinical studies of therapies targeting the α7 nAChR alone have demonstrated little success, suggesting a more complex pharmacological landscape or potential interference by prior drug treatment (Gill et al., 2014). A recent meta-analysis of 13 randomized controlled trials of α7 nAChR agonist drugs found no significant impact on cognitive symptoms and a minimal impact on negative symptoms of SCZ across trials (Recio-Barbero et al., 2021). Additionally, a double-blinded, placebo-controlled trial of the α7 nAChR positive allosteric modulator AVL-3288 failed to improve auditory P50 potential suppression as well as assessments of cognitive and negative symptoms in non-smoking SCZ patients (Kantrowitz et al., 2020). Further experiments employing regional, nAChR subtype–specific modulation could better elucidate the precise mechanism by which systemic NIC reduces both vHipp and VTA DA hyperactivity in MAM rats and how such nicotinic receptor mechanisms may translate to human patients. Importantly, the potential for normalization of vHipp activity has implications for effects on projection regions beyond the VTA, potentially impacting other symptom categories (Grace, 2016). Further studies are necessary to better clarify the potential mechanistic link between NIC effects on measurable behavior and neuronal activity.

The results of these experiments suggest that administration of a moderate dose of NIC can enhance measures of sensory gating and recognition memory and normalize VTA and vHipp neuronal activity in the MAM model of SCZ. These findings lend support to a self-medication hypothesis behind increased smoking in SCZ by demonstrating that NIC may alleviate deficits associated with a SCZ-like endophenotype in rats. While these experiments illustrate the potential utility of the MAM model in further exploring nicotinic mechanisms of cognitive enhancement in SCZ, they cannot provide direct insight regarding how these cognitive effects of NIC may motivate patients to smoke more. When surveyed, SCZ smokers were not more likely than controls to cite cognitive enhancement as a motivator for smoking, though relief of negative affect was more consistently reported among patients (Forchuk et al., 2002; Galazyn et al., 2010). A self-medication hypothesis could thus encompass a general sense of "feeling better," rather than discrete effects on cognitive performance, as a motivator for increased smoking in SCZ. Nonetheless, these findings offer evidence linking specific elements of SCZ neurophysiology and behavior modeled by GD17 MAM to a body of evidence suggesting the potential of nicotinic agonists in improving cognitive dysfunction in SCZ. Our experiments also confirmed that, like α7-specific agonists, systemic NIC administration can normalize elevated VTA DA population activity and vHipp hyperactivity observed in MAM rats. Although our experiments did not directly link our neurophysiological and behavioral observations, these findings provide potential evidence that normalization of vHipp output could impact activity in projection regions beyond the VTA and thus normalize behavioral dysfunction. Further knowledge in this area may also offer insight into the actions of NIC

on cognitive deficits in SCZ and as such guide the development of therapeutic approaches to improve these deficits. Our neurophysiological and behavioral findings provide valuable evidence to the growing understanding of the effects of NIC in SCZ developed through studies in animal models.

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Interest Statement

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