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Polysubstance addiction vulnerability in mental illness: Concurrent alcohol and nicotine self-administration in the neurodevelopmental hippocampal lesion rat model of schizophrenia

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Abstract

Multiple addictions frequently occur in patients with mental illness. However, basic research on the brain-based linkages between these comorbidities is extremely limited. Toward characterizing the first animal modeling of polysubstance use and addiction vulnerability in schizophrenia, adolescent rats with neonatal ventral hippocampal lesions (NVHLs) and controls had 19 weekdays of 1 hour/day free access to alcohol/sucrose solutions (fading from 10% sucrose to 10% alcohol/2% sucrose on day 10) during postnatal days (PD 35-60). Starting in adulthood (PD 63), rats acquired lever pressing for concurrent oral alcohol (10% with 2% sucrose) and iv nicotine (0.015 mg/kg/injection) across 15 sessions. Subsequently, 10 operant extinction sessions and 3 reinstatement sessions examined drug seeking upon withholding of nicotine, then both nicotine and alcohol, then reintroduction. Adolescent alcohol consumption did not differ between NVHLs and controls. However, in adulthood, NVHLs showed increased lever pressing at alcohol and nicotine levers that progressed more strongly at the nicotine lever, even as most pressing by both groups was at the alcohol lever. In extinction, both groups showed expected declines in effort as drugs were withheld, but NVHLs persisted with greater pressing at both alcohol and nicotine levers. In reinstatement, alcohol reaccess increased pressing, with NVHLs showing greater nicotine lever activity overall. Developmental temporal-limbic abnormalities that produce mental illness can thus generate adult polydrug addiction vulnerability as a mechanism independent from putative cross-sensitization effects between addictive drugs. Further preclinical modeling of third-order (and higher) addiction-mental illness comorbidities may advance our understanding and treatment of these complex, yet common brain illnesses.

KEYWORDS

addiction, alcohol, comorbidity, mental illness, neurodevelopmental, nicotine

1 | INTRODUCTION

Complex comorbidities of psychiatric illness and substance use disorders are mainstream brain health conditions, frequently involving serious illnesses such as schizophrenia where about half of patients have alcohol and/or illicit substance disorders and more than 75% are nicotine-addicted.[1-5] Although often termed "dual diagnosis" implying comorbidity of just two disorders (ie, one mental illness and one addiction), many

dual diagnosis cases involve higher order combinations of multiple addictions with one or more mental illnesses.[6-8] These "high order" dual diagnosis cases represent significant treatment challenges not only because these patients are sicker but also because behavioral health care remains largely fragmented into segregated mental health vs addiction services that are unable to provide integrated care.[9-11]

To advance prevention and treatment of third (and higher) order dual diagnosis conditions, more research is needed to better understand and counteract biological mechanisms that link severe mental illnesses like schizophrenia and polyaddiction vulnerability.[12,13] The present study pursues this goal preclinically by examining concurrent polysubstance self-administration in a widely studied and well-validated neurodevelopmental animal model of schizophrenia. Specifically, we examined concurrent adult self-administration of both alcohol and nicotine in the neonatal ventral hippocampal lesion (NVHL) model, which is produced by delivery of axon-sparing neurotoxic lesions to 7-day-old rat pups.[14] Both alcohol and nicotine are commonly used in the general population, and by schizophrenia patients in addictive patterns at rates at least two times greater than in the general population.[15-18] Thus, our study design provides a first animal modeling approach to a commonly encountered mental illness/polysubstance combination that allows for measurement of multidrug addiction vulnerability in the context of concurrent polydrug use.

The applicability of the NVHL model in this approach is suggested by an accumulation of over 100 studies characterizing the mental illness and/or the addiction vulnerability features of the model.[19] NVHLs generate a developmentally progressive syndrome that encompasses cognitive and negative symptom domains of human schizophrenia with positive-range symptoms that worsen after adolescence.[19] The model also produces frontal cortical-striatal-limbic circuit dysfunction that mimics core histopathological, neuroimaging, and neurochemical features of schizophrenia, including markers of prefrontal dysfunction (ie, "hypofrontality") and striatal network hypersensitivity to the effects of mesolimbic dopamine release.[19-24]

These same biological attributes likely underpin impairments of decision making and impulse control in the NVHL model that emulate human endophenotypes of addiction vulnerability measured before drug exposure.[25-27] With drug exposure, NVHLs show acceleration/amplification of the addictive disease process in a non-drug specific way as measured by both experimenter delivered (ie, behavioral sensitization) to cocaine, alcohol, or nicotine[28-30] and self-administration (ie, instrumental learning reinforcement) to all of these same three drugs[31-36] and methamphetamine.[37]

This present study is the first to compare NVHL vs SHAM-operated (healthy) rats in the acquisition of instrumental responding for any two drugs (in this case alcohol and nicotine) self-administered concurrently, followed by tests of drug seeking during extinction and drug-induced relapse. To increase the likelihood that rats would concurrently self-administer both drugs in adulthood, our design was informed by the capacity of adolescent drug exposure to enhance adult addiction risk.[38] Specifically, given our estimation that the rats might be slower to acquire operant responding for access to oral alcohol compared with iv nicotine delivery, and given prior work by Jeanblanc et al, showing that adult alcohol consumption is amplified in NVHL rats after adolescent alcohol drinking,[34] we first exposed both NVHL and SHAM rats to free-access alcohol drinking during adolescence, followed by concurrent instrumental operant acquisition to both alcohol and nicotine in adulthood.

2 | METHODS

2.1 | Subjects and neonatal surgeries

Sprague Dawley litters born from rats arriving 14 to 17-day gestation (Harlan, Indianapolis) were culled to males on postnatal day (PD) 3 in preparation for surgeries on PD 7. Thirty-six pups weighing 16 to 19 g were randomized evenly to NVHL or SHAM surgeries as detailed in the previous study.[39] Briefly, ibotenic acid (3.0 μg; Sigma) in 0.3 μL artificial CSF (or aCSF only for SHAMs) was delivered under hypothermic anesthesia via infusion into the ventral hippocampus bilaterally (A/P −3.0 mm, M/L ± 3.5 mm, DV −5.0 mm from bregma). Pups were reared under standard conditions until weaning (PD 21), then housed in lesion-like pairs until adulthood, when they were individually housed following jugular venous catheterization. Surgical and experimental procedures (Figure 1) were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and Indiana University IACUC-approved protocols.

2.2 | Drug preparation and adolescent alcohol exposure

Nicotine hydrogen tartrate salt (Sigma) was dissolved in 0.9% normal saline to a stock solution of 0.25 mg/mL free base and adjusted to pH of 7.4.[40] For iv self-administration, doses were prepared daily on a per rat basis from stock to achieve 0.015 mg/kg/infusion. Stock also

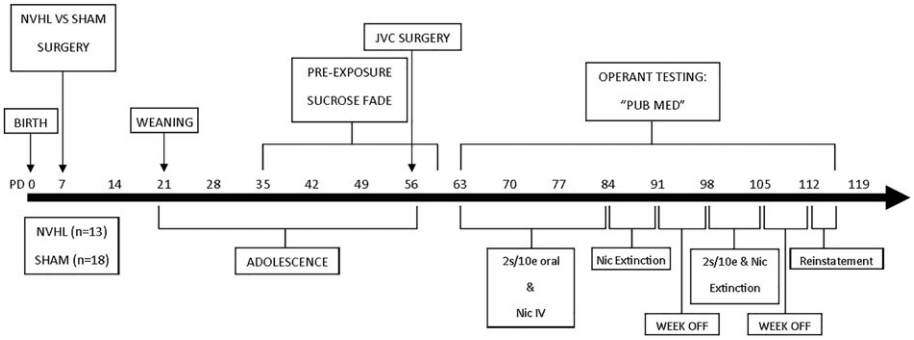

FIGURE 1 Surgical and experimental timeline

provided subcutaneous injections at a volume of 1 mL/kg during reinstatement.

Sucrose/alcohol solutions were prepared for the adolescent preexposure and adult self-administration experiments as delivered via sipper tube or trough/mechanical dipper, respectively. The preexposure sucrose/alcohol fade was based on protocols in the previous studies[31,41] (which reliably produce alcohol consumption leading to a final solution that has carbohydrate and alcohol concentrations comparable with human alcoholic beverages. Over 19 days, adolescent rats had access to the following: 3 days of 10% sucrose, 2 days of 10% sucrose/2% ETOH (10s/2e), 2 days of 10s/5e, 1 day of 10s/10e, 1 day of 5s/10e, and then 2s/10e for 10 more sessions. This regimen was delivered over a 4-week (Monday-Friday) schedule (PD 35-60), except for on the fourth Monday (PD 56) when rats underwent iv catheterization surgeries. Rats had access to these solutions (via sipper tubes) for 1 hour/day in Plexiglas cages away from their home cages.

2.3 | Jugular catheterization surgery

On PD 56, subjects underwent jugular venous catheterization as described previously,[33] with Silastic tubing (Dow Corning, Midland, MI) threaded into the right jugular vein coursing subcutaneously over the shoulder to exit the back via 22-gauge cannula (Plastics One, Roanoke, VA). To maintain patency, catheters were flushed before and after operant sessions and once daily on weekends with 0.3 mL of 20 u/mL heparinized saline containing 0.13 mg/mL gentamicin. Patency was verified after Friday operant sessions by pushing 0.1 mg/0.1 mL iv of methohexital sodium (McKesson, USA) which produces a brief loss of consciousness. Rats with failed or infected catheters were excluded from the experiment.

2.4 | Operant coself-administration of oral alcohol and IV nicotine

Concurrent self-administration was conducted in Med Associates chambers (St. Albans, VT) interfaced with Med PC software that controlled lighting and drug deliveries while recording instrumental activity. Our eight units, modified specifically for concurrent instrumental delivery of alcohol and nicotine (as in simulation of the traditional "Pub"), were termed "Pub-Med" and equipped with three nonretractable levers across the right wall of the chamber with only a house light on the opposing (left) wall. The first lever (left most, from the rat's perspective) activated a magazine with a 0.1-mL dipper cup that retrieved 2s/10e solution from a trough; the second lever (middle, "blank") was completely inactive; the third lever (rightmost) initiated an iv infusion that delivered 0.015 mg/kg of nicotine. Cue lights were positioned above the levers and the magazine delivering the alcohol dipper (located between alcohol and blank levers).

Self-administration sessions in Pub-Med began on PD 63 for 5 days/week (M-F) for seven and a half weeks. To promote exploratory behavior for the operant procedure, rats were food deprived 24 hours preceding the first operant session and remained food restricted to greater than 85% of PD 63 body weight with delivery of two to three pellets daily of rat chow after sessions through the acquisition phase. Over this 3-week phase (15 × 1-h sessions), both drug-paired levers delivered reinforcers on an FR1 schedule. The house light was on for the duration of the sessions. Presses on the alcohol lever activated the cue lights above the lever and the magazine for the 10-second dipper presentation bearing 2s/10e solution. Presses on the alcohol lever during this 10-second interval had no consequences and were recorded as timeout presses. Active presses on the nicotine lever delivered a 0.015 mg/kg/infusion over 3 seconds followed by a 7-second timeout phase. The cue light above the nicotine lever was on for 10 seconds including during the infusion and timeout phase; more nicotine lever presses during this phase had no consequences but were recorded as timeout presses. Presses on the middle blank lever were also recorded. All levers were able to operate independently, and both drugs could be consumed simultaneously or in any back and forth pattern. Cue lights and software programming remained consistent through all phases of self-administration (Figure 1) including acquisition (sessions 1-15), nicotine-only extinction when 2s/10e was still available (sessions 16-20), during extinction from both nicotine and alcohol (sessions 21-25), and over three sessions of reinstatement (sessions 26-28). Although it would have been informative to extinguish either nicotine or alcohol first, we chose nicotine first, since the drug (being delivered iv) was likely more reinforcing than the alcohol. Tail bloods were collected 30 ± 10 minutes of the sessions on Fridays of weeks 1 and 2 (sessions 5 and 10) of acquisition to confirm alcohol consumption. Rats were given a week off from Pub-Med testing after both the nicotine and nicotine/alcohol extinction phases. For reinstatement, on session 26, rats were given a 1 mL/kg subcutaneous injection of saline 30 minutes prior to the start of the session. On session 27, rats were given a 0.25 mg/kg sc nicotine injection 30 minutes prior. For the last session, rats were again given nicotine 30 minutes prior and troughs were filled with 2s/10e.

2.5 | Histological lesion verification

Following Pub-Med sessions and sacrifice, brains were removed whole and cryostat cut into 40-μM coronal sections through the rostral-caudal extent of the hippocampus. Mounted sections were fixed and 0.5% thionin stained. Microscopic examination of both lesioned and SHAM brains was performed blind to behavioral data. Rats showing bilateral evidence of atrophy, paucity of nuclei, and cellular disarray in the ventral hippocampus with some lateral ventricular enlargement were included. Brains with unilateral damage, dorsal hippocampal damage, or direct damage encompassing structures adjacent to the ventral hippocampus were excluded from the study.[39] Five lesioned rats were excluded yielding final totals of 13 NVHL and 18 SHAMS rats (Figure 2).

2.6 | Data analysis

Analyses of adolescent drinking and adult self-administration data generally utilized mixed model repeated measures ANOVAs with lesion status as the main independent factor and alcohol consumption or bar pressing as dependent variables. Adolescent drinking (10

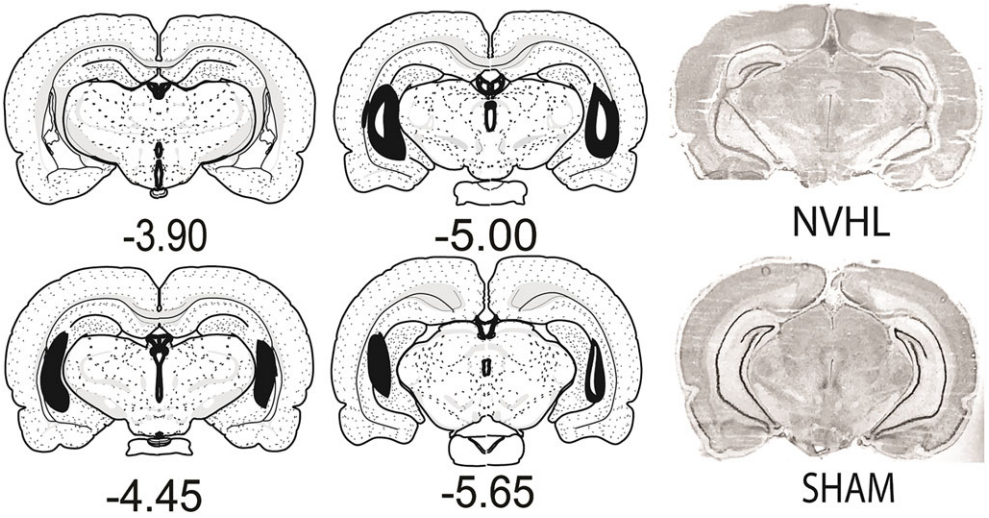

FIGURE 2 Brain mapping of lesion extent. Maps on left show the range of lesion impact across all N = 13 NVHL rats included in the study at sections AP relative to Bregma (maps adapted from Swanson, LW (2004) brain maps: Structure of the rat Bain, 3rd ed., New York, Elsevier). Largest extent of lesions in the group are shown in black with smallest lesions shown as white insets (eg, at −5.00). Right column micrographs show a medium-sized lesion in the NVHL group vs a SHAM rat

sessions at the 2s/10e concentration), adult acquisition (15 sessions), nicotine extinction (5 sessions), alcohol and nicotine extinction (5 sessions), and reinstatement (3 sessions) were each examined independently with separate analyses on each lever. Active presses and timeout or total presses were analyzed separately. For acquisition sessions, simple post hoc *t* tests where applied to compare NVHL vs SHAM pressing on each day to assist with interpretation of the initial ANOVAs that revealed simple lesion or lesion × day interactions. All significant statistical results (assumed at $P < 0.05$) and informative negative results are reported with group mean ± SEM throughout.

3 | RESULTS

3.1 | Adolescent drinking and adult acquisition of coself-administration

Across the final 10 weekday sessions of adolescent drinking (PD 46-60) at the 2s/10e concentration, rats significantly increased their consumption (day: $F(9, 261) = 6.3$, $P < 0.001$) so that alcohol intake increased from 0.13 ± 0.03 mg ETOH/kg rat on day 1 to 0.47 ± 0.08 g/kg on day 10. There was no lesion-based difference in consumption (lesion: $F(1, 29) = 0.8$, NS) or lesion × day interaction ($F(9, 261) = 0.8$, NS).

Over the 15 concurrent alcohol (2s/10e)/nicotine acquisition sessions, active presses on the alcohol lever (Figure 3A) increased for all animals (day: $F(14, 406) = 10.1$, $P < 0.00$), with NVHLs showing greater active pressing (lesion: $F(1, 29) = 7.7$, $P < 0.01$) but no lesion × day interaction $F(14, 406) = 0.7$, NS), even as NVHL and SHAMs showed the same level of active presses on session 1. Timeout responding on the alcohol lever (Figure 3B) also increased then stabilized over the 15 sessions (day: $F(14,406) = 6.6$, $P < 0.001$), but there were no lesion ($F(1, 29) = 1.9$, NS) or lesion × day interaction ($F(14, 406) = 0.6$, NS). To confirm that animals were actually drinking alcohol presented to them via active alcohol lever presses, tail blood alcohol levels were analyzed using linear regressions between the number of dipper presentations (*x*-variable) and tail blood alcohol levels (*y*-variable). After the fifth session, 12 NVHL and 13 SHAM rats yielded adequate blood samples showing a significant linear relationship (($F(1, 24) = 16.8$, $P < 0.001$); $R = 0.65$; $y = 0.69x + 5.2$) (Figure 4A). After the 10th session, a better yield of tail bloods from all 13 NVHL and 18 SHAMs also showed a significant relationship between blood alcohol levels and active alcohol lever presses (($F(1, 30) = 6.9$, $P < 0.05$); $R = 0.44$; $y = 0.35x + 6.3$) (Figure 4B).

Active nicotine lever presses also increased steadily over the 15 acquisition sessions (day: $F(14, 406) = 16.5$, $P < 0.001$), after NVHL and SHAM rats started out at similar low levels on day 1 (Figure 3C). NVHLs also showed greater overall active nicotine lever presses (lesion: $F(1, 29) = 10.9$, $P < 0.01$) with a significant day × lesion interaction ($F(14,406) = 2.2$, $P < 0.01$) that, as suggested by simple post hoc comparisons on each day, was generated by a progressive widening of group differences expressed over the final eight sessions. Timeout responding on the nicotine lever showed similar patterns but with less statistical strength in terms of group differences (Figure 3D) with overall increases in pressing (day: $F(14, 406) = 5.3$, $P < 0.001$), where NVHLs produced more presses overall (lesion: $F(1, 29) = 7.7$, $P < 0.05$) and in a significant lesion × day interaction ($F(14, 406) = 1.8$, $P < 0.05$).

Analysis of responding on the blank lever (Figure 3E) also showed more subtle but still significant increases in lever responding for all rats across the 15 sessions (day: $F(14, 406) = 2.7$, $P < 0.001$). There was also greater NVHL pressing (lesion: $F(1, 29) = 5.7$, $P < 0.05$) without the day × lesion interaction ($F(14, 406) = 1.1$, NS) on the blank lever. Examination of the fractions of total activity directed at each lever (Figure 5) shows that relative levels of responding evolved on all three levers (alcohol (days: $F(14, 406) = 8.7$, $P < 0.001$), nicotine (day: ($F(14, 406) = 3.0$, $P < 0.001$), blank ($F(14, 406) = 5.6$, $P < 0.001$)) with the proportion of alcohol responding growing faster than nicotine responding over the first 5 days, then stabilizing at about 2:1 ratios (alcohol to nicotine) over days 5 to 15 with blank lever

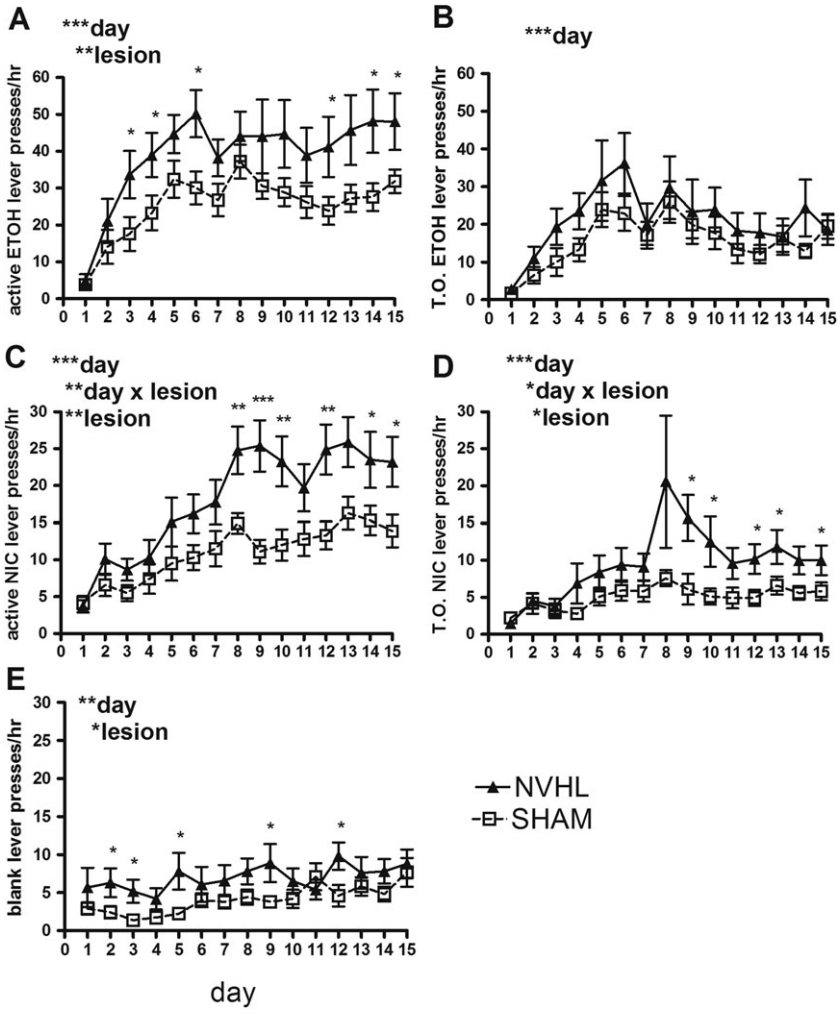

FIGURE 3 Acquisition of concurrent instrumental self-administration of oral alcohol and iv nicotine. (A) Active alcohol (ETOH) lever hits presenting access to 0.1 mL 2s/10e solution were greater in NVHL rats (**), (B) with no group differences in timeout (T.O.) responding on the alcohol lever. On the nicotine (NIC) lever, (C) active hits delivering 0.015 mg/kg nicotine were greater in NVHL rats (**), accompanied by greater across-session growth of lever pressing in NVHLs (** day × lesion). (D) T.O. responding on the NIC lever was also greater in NVHLs overall and in terms of lesion specific growth but with less statistical strength (*). (E) Blank lever hits were also elevated in NVHLs overall (*) but with less strength than at alcohol and nicotine levers, and with no day × lesion interaction. All bars reflect mean ± SEM with asterisks above bars representing simple *t* test comparisons by lesion status on that day. All asterisks represent degree of statistical significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

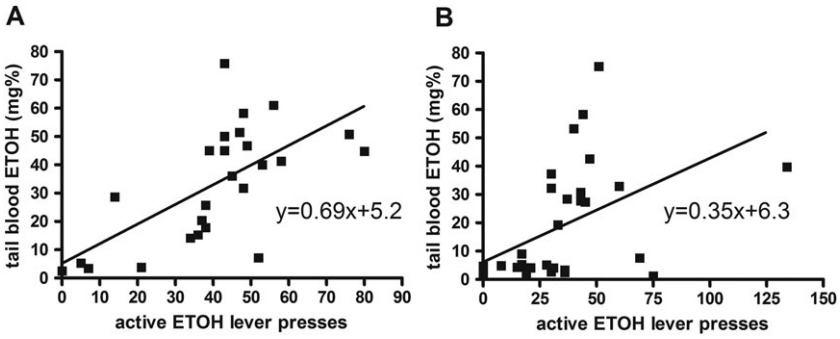

FIGURE 4 Linear correlations between active ETOH lever pressing (cups presenting 0.1 mL of 2s/10e solution) and tail blood alcohol levels collected 30 ± 10 minutes after sessions on Fridays of weeks 1 and 2 (sessions 5 and 10). After session 5 (A), adequate samples were drawn from n = 25 rats yielding a significant liner correlation ($F(1, 24) = 16.8$, $P < 0.001$), and after session 10 (B), all 31 rats yielded a significant correlation ($F(1, 30) = 6.9$, $P < 0.05$)

pressing being relatively extinguished. NVHLs did not differ from SHAMs in overall proportions of type of lever responses but did show a significant growth in the relative proportion of presses at the nicotine lever (day × lesion: $F(14, 406) = 1.9$, $P < 0.05$).

3.2 | Single and polydrug extinction

In sessions 16 to 20 with nicotine but not alcohol delivery withheld, there was no overall change in alcohol lever responding examined as active presses (Figure 6A; day: $F(4, 116) = 1.0$, NS), or total alcohol lever presses (active + timeout hits are examined for better comparison with total nicotine lever presses, which has no active vs timeout component) (Figure 6B; day: $F(4, 116) = 0.7$, NS)). However, NVHLs continued to show greater alcohol lever responding for both active (lesion: $F(1, 29) = 6.6$, $P < 0.05$) and total presses ($F(1, 29) = 6.3$, $P < 0.05$). In contrast, there was a significant decline in total lever pressing on the nicotine lever (Figure 6C) consistent with extinction (day: $F(4, 116) = 8.1$, $P < 0.001$) while NVHL rats continued to press for nicotine to a greater extent (lesion: $F(1, 29) = 11.1$, $P < 0.01$) than SHAMs across these sessions, without a day × lesion interaction ($F(4, 116) = 0.8$, NS)).

In sessions 21 to 25, with delivery of both alcohol and nicotine denied, total alcohol lever pressing (Figure 7A) declined significantly consistent with extinction (day: $F(4, 116) = 19.2$, $P < 0.001$), while NVHL rats persisted in pressing for ETOH at greater rates than

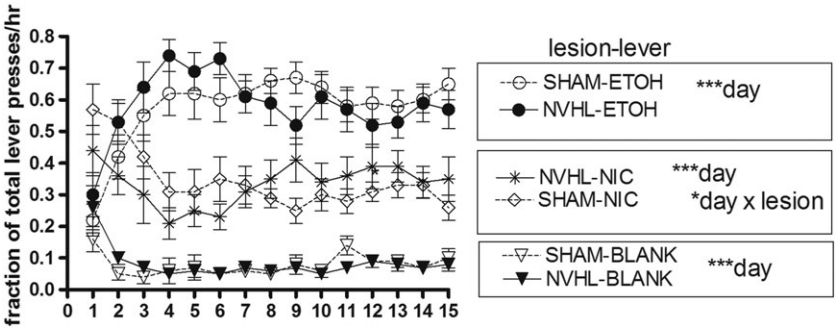

FIGURE 5 Reexamination of acquisition responding as relative fractions of activity on alcohol vs nicotine vs blank levers. The fractions of total pressing evolved highly significantly on all three levers (***day), settling into 6:3:1 ratios (ETOH/NIC/BLANK) by day 5. There was no overall group difference in preference for lever, although NVHLs showed a significant time progression of responding preference on the nicotine lever (*day × lesion). All bars reflect mean ± SEM (*$P < 0.05$; ***$P < 0.001$)

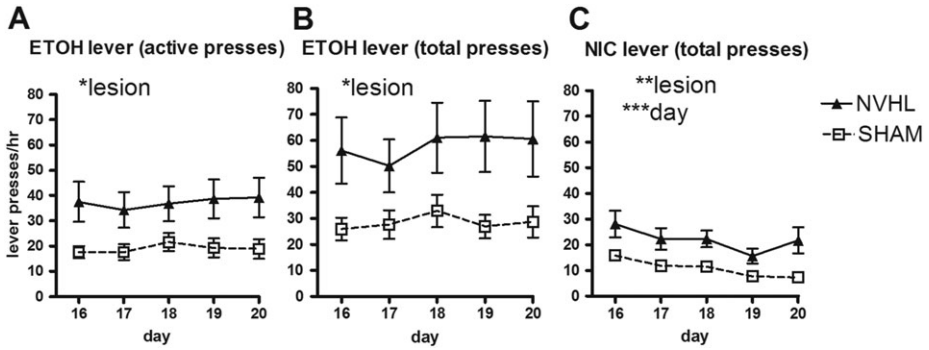

FIGURE 6 Extinction from nicotine only with continuation of alcohol access. NVHL rats continued to press more frequently on the ETOH lever (*) in terms of both active (A) and total (active + T.O.) hits (B). Meanwhile, overall responding extinguished on the NIC lever (***day) but with NVHL rats continuing to press for nicotine (**). All bars reflect mean ± SEM (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

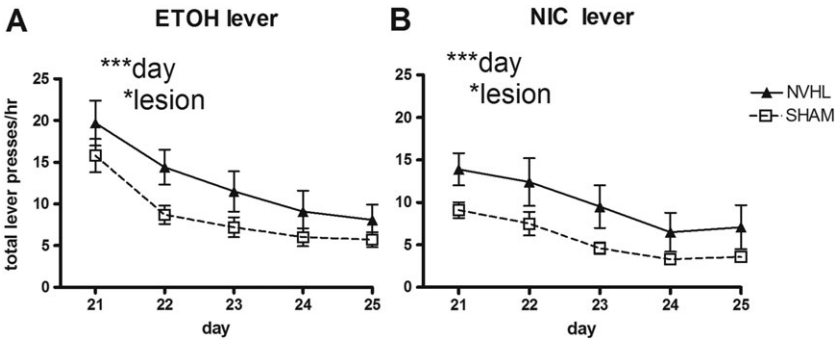

FIGURE 7 Extinction from nicotine and alcohol access. (A) Total hits on the ETOH lever declined relatively precipitously (***day) compared with (B) on the NIC lever (*day) which had already been largely extinguished. NVHLs continuing to press more overall on both ETOH and NIC levers (*). All bars reflect mean ± SEM (*$P < 0.05$; ***$P < 0.001$)

SHAMs (lesion: $F(1, 29) = 4.6$, $P < 0.05$) without a day × lesion interaction ($F(4, 116) = 0.4$, NS). On the nicotine lever (Figure 7B), in continuation of patterns observed in the prior nicotine-only extinction sessions (Figure 6), rats continued to show ongoing declines in total pressing (day: $F(4, 116) = 13.5$, $P < 0.001$), with NVHL rats still pressing more for nicotine overall (lesion: $F(1, 29)$: 5.4, $P < 0.05$; day × lesion: $F(4, 116) = 0.28$, NS). Analysis of blank lever pressing during the final five extinction sessions showed ongoing declines in low-level pressing (day: $F(4, 116) = 3.3$, $P < 0.05$; means of all rats from 7.4 ± 1.0 presses/h (session 21) to 4.5 ± 1.2 presses/h (session 25), with no differences based on lesion (lesion: $F(4, 29) = 1.7$, NS; day × lesion: $F(4, 116) = 0.45$, NS)). This blank lever pressing differed from blank lever pressing when ETOH was still being delivered during nicotine-only extinction (sessions 16-20). Over these sessions, although overall blank lever responding was also quite low and still extinguishing (day: $F(4, 116) = 4.9$, $P < 0.01$), NVHL rats were pressing more (lesion: $F(1, 29) = 14.4$, $P < 0.01$) and showed steeper overall declines in responding (day × lesion: $F(4, 116) = 2.7$, $P < 0.05$), such that NVHLs declined from 9.9 ± 2.2 presses/hour (session 16) to 4.0 ± 0.8 presses/hour (session 20), compared with SHAMs with 3.8 + 0.8 (session 16) and 2.5 ± 0.7 presses/hour (session 20).

3.3 | Single and polydrug reinstatement of drug seeking

Across all three reinstatement sessions (Figure 8), there was no change in blank lever pressing (day: $F(2, 58) = 2.5$, NS) or lesion-based differences in blank lever pressing (lesion: $F(1, 29) = 1.8$, NS; day × lesion: $F(2, 58) = 1.8$, NS). However, there was an overall increase in total lever pressing on the alcohol lever (day: $F(2, 58) = 26.5$, $P < 0.001$) as expected, with substantial increases when alcohol became available on day 3. These changes were not accompanied by lesion-based differences in alcohol lever presses (lesion: $F(1, 29) = 0.07$, NS;

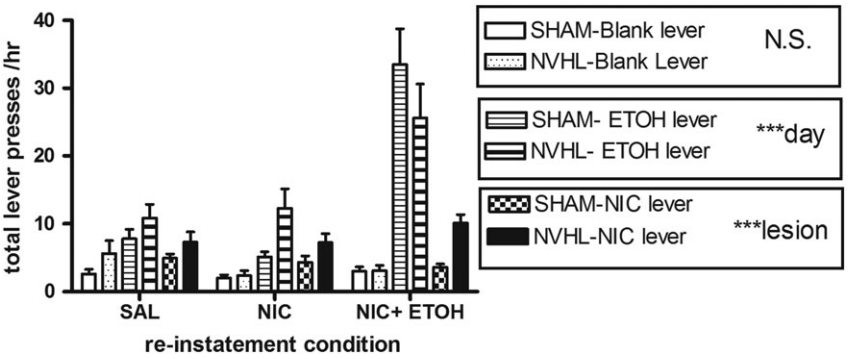

FIGURE 8 Nicotine and alcohol reinstatement sessions. No lesion-based differences emerged on the blank lever, regardless of saline (SAL) vs nicotine (NIC) preinjection condition vs return of alcohol access with nicotine preinjections (NIC). Pressing on the ETOH lever increased strongly (***day) with return of alcohol access but not differentially so by lesion. NVHLs persisted in showing increased activity on the NIC lever (***lesion) regardless of reinstatement condition. All bars reflect mean ± SEM (***$P < 0.001$)

day × lesion: $F(2, 58) = 2.9$, NS). On the nicotine lever, NVHLs continued to press more (lesion: $F(1, 29) = 17$, $P < 0.001$), without substantial increases across sessions overall (day: $F(2, 58) = 0.7$, NS) or according to lesion status (day × lesion: $F(2, 58) = 2.9$, NS).

4 | DISCUSSION

This study is to our knowledge the first to demonstrate concurrent instrumental oral alcohol and iv nicotine self-administration in a neurodevelopmental model of mental illness, and to show that addictive behaviors with respect to both drugs are simultaneously increased by the mental illness model. Le et al have previously shown that healthy Wistar rats will concurrently self-administer oral alcohol and iv nicotine[42] and that in Long Evans rats, initial self-administration of nicotine will enhance subsequent self-administration of alcohol.[43] The latter finding is consistent with a "cross-sensitization" or "gateway effect," whereby use of, or addiction to, one drug biologically predisposes to addiction to one or several others via shared or synergistic pharmacobiological effects (eg, in the mesolimbic reward pathways).[44] While providing a compelling explanation for the occurrence of polysubstance use disorders, eg, where rates of nicotine addiction are twofold to threefold higher in patients with alcoholism than in the general population,[16] the present findings are among the first to suggest an additional causal dynamic: The cooccurrence of two drugs used in addictive patterns may be caused by their shared vulnerability or "gravitation" toward a third pathological entity—the mental illness. This possibility has been preliminarily supported by prior work in the NVHL model showing that it confers elevated addiction vulnerability to both nicotine and alcohol, completely independently of one another,[31,32,34] whereas the present study confirms it in the context of concurrent self-administration of both drugs.

It is possible that both the cross-sensitization effect and mental illness-induced vulnerability to addiction may simultaneously, biologically attract all these pathologies together causing high-order dual diagnosis cases that are now routinely encountered in behavioral health care settings.[8] Notably, the present study does not rule out the possibility that a cross-sensitization effect between alcohol and nicotine was also in play, nor was it designed to test which pathological attraction, the "drug-drug" or the "drug-mental illness" one, is greater. Future animal studies are needed to dissect and characterize the relative strengths of these causal dynamics, including the intriguing possibility that mental illness could biologically enhance drug-to-drug sensitization. The majority of work looking at how the NVHL model biologically worsens the addiction process has been limited to cocaine. These studies have shown that while the NVHL model does not increase drug-induced DA release into the ventral striatum as compared with healthy animals,[22] the cumulative neuroplastic and behavioral effects of drug-induced DA release are accentuated by abnormal neuronal activity[21] and gene expression patterns[20] present in both the prefrontal cortex and dorsal striatum of NVHL rats. So, to the extent that alcohol and nicotine both exert cocaine-like reinforcing effects in schizophrenia[32,45] via their shared effects on increasing mesostriatal DA transmission, it is plausible to speculate that both their independent and synergistic (eg, drug-drug sensitizing) neuroplastic effects may be expressed and amplified by the NVHL model—postsynaptic to DA transmission within prefrontal cortex and striatal networks. Consistent with this, GABAergic-interneuron control of prefrontal cortical networks (eg, required for adult-age capacities for working memory and impulse control) requires proper maturation during adolescence, and is modulated by mesocortical DA afferents.[46,47] NVHLs disrupt the development of these complex regulatory interactions leading to impaired DA modulation of prefrontal GABAergic interneurons in adulthood, and other physiological signs of cortical incoherency in which both GABA and glutamatergic neurons are implicated.[24,48,49] Given evidence presented here and in prior studies that addiction vulnerability of NVHLs is more fully expressed after adolescence, and correlates with deficits of working memory and impulse control,[27,32] a complex array of disruptions of information processing and neuroplasticity involving both excitatory and inhibitory neurotransmission within cortical-striatal networks likely contribute to both mental illness symptoms and nondrug-specific susceptibility to addiction.

A key design feature of the present study is that rats had a free-access drinking regimen of a sucrose/alcohol solution during adolescence as informed by Jeanblanc et al, to increase the likelihood of successful acquisition of concurrent instrumental self-administration of both drugs in adulthood.[34,38] This approach was also undertaken to help avoid the possibility that heavy use of one drug in the operant boxes could actually drive down use of another drug as has been observed under certain circumstances between alcohol and nicotine[43] in healthy rats. Our findings suggest that our approach worked, while replicating prior findings showing that although the NVHL model does not cause increased alcohol consumption during adolescence, the

alcohol addiction vulnerability phenotype of NVHLS is expressed in adulthood.[34] Similarly, we have seen the same kind of effect with nicotine: Behavioral sensitization to nicotine is not increased in adolescent NVHLs, although it is in adult NVHLs, who also show increased nicotine self-administration compared with SHAMs, regardless of adolescent nicotine exposure.[28,32] Notably, because all rats were exposed to alcohol solutions in adolescence, this study was not able to determine if this exposure was necessary for or caused differential expression of the adult NVHL vs SHAM phenotypes.

The use of a three-lever system in the present experiments with one blank lever, and timeout phases on both the alcohol and nicotine levers, allowed us to examine NVHL-model differences in the specificity of drug pursuit and effort. During acquisition, NVHL-based differences from SHAMs were greater for active lever as compared with timeout responding with respect to both drugs, and as compared with blank lever hitting. These patterns indicate that NVHLs specifically increased pressing that was most certain to deliver drug, but simultaneously some degree of NVHL-induced nonspecific overflow of pressing (on the nicotine timeout and blank lever) did occur. Even so, this nonspecific pressing in NVHLs was strongest for nicotine-lever timeout responding, which emerged in the last half of the acquisition series when nicotine intake reached the greatest levels, whereas NVHL difference in blank hitting became more infrequent with more sessions.

The behavioral economy of overall lever responding was remarkably similar between the groups with rats settling into patterns by day 4 of acquisition where 50% to 75% of all presses were on the alcohol lever, 20% to 40% were on the nicotine lever, and <10% were on the blank lever. It is not entirely clear why the alcohol lever was pressed more frequently than the nicotine lever overall for both active and timeout presses, although active nicotine presses were guaranteed to deliver a 3-second iv infusion, whereas active alcohol lever presses just presented the cup of alcohol, still requiring the animal to find and drink from it. This difference may have produced more relative effort to access the alcohol. Also, since all rats were already experienced with alcohol (in adolescence) but not nicotine, they may have already been more motivationally sensitized to alcohol compared with nicotine. Notably, in anecdotal observation through viewers into the chambers on the first acquisition day, we saw rats immediately maneuvering to the trough where the alcohol was available, presumably drawn by alcohol odor cues. Despite all this, NVHL rats did show a significant growth in the percentage of activity on the nicotine lever compared with SHAMs (but not on the other levers), which led to more robust NVHL-nicotine findings in the extinction and reinstatement sessions.

Over the first extinction series, where nicotine (but not alcohol) delivery was denied, group differences between NVHLs and SHAMs were statistically more significant on the nicotine ($P < 0.01$) compared with alcohol lever ($P < 0.05$), while significant differences between session declines in responding were only seen on the nicotine lever. Then, during the next extinction series when both drugs were denied, significant declines in both levers were obtained, with the NVHL rats maintaining increased efforts on both levers. During reinstatement, NVHL activity on the nicotine lever was exclusively higher (compared with the other levers) pervasively across sessions, whereas return of alcohol access significantly boosted alcohol lever activity for all rats regardless of lesions status. Somewhat unexpectedly, nicotine injections introduced in the second reinstatement session did not significantly increase nicotine lever responding compared with levels on the first (saline injection day). It is possible that the nicotine dose used (0.25 mg/kg/sc) was not high enough to elicit a strong reinstatement response specific to NVHLS as we have seen by using a 0.5 mg/kg dose reported previously.[27] In any event, the acquisition, extinction, and reinstatement data collectively suggest that in the context of the adolescent alcohol preexposure, the adult addiction vulnerability phenotype expressed in the NVHL model is generalizable to both alcohol and nicotine but occurs with greater robustness with nicotine. This interpretation should be qualified by the fact that the alcohol in this paradigm had to be orally consumed, whereas the nicotine was iv injected. This difference created reliability, workload, and pharmacokinetic differences for the animals in terms of how the two drugs arrived in their brains, which all could be assumed to decrease the relative addictive potential of alcohol compared with nicotine in this paradigm. Moreover, it is generally accepted that nicotine is relatively more addictive than alcohol, and so, our findings are interpretable as indicating that NVHLs increase addiction risk in a way that is more readily expressed with more addictive drugs.

In summary, the present findings, while representing at least the second published replication showing increased addiction vulnerability to either nicotine[27,32] or alcohol[31,34,36] in the NVHL model, are the first to show the comorbidity of these addiction vulnerability phenotypes when both drugs are used concurrently. A huge variety of alternative study designs may be pursued based on the mental illness/polyaddiction model approach described here to investigate the interactive pathologies of these conditions on neurobiology, cognition, and motivation. While providing a platform to preclinically investigate novel preventative and treatment approaches for third and higher order dual diagnosis patients, these findings point to a fundamental connection between mental illness and multiple addictions that should translate clinically to greater integration of professional training, treatment, and research on these complex comorbidities.[6,12]

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CONFLICT OF INTEREST

RAC has advisory and/or creative contracts with Enfoglobe (Medical Education and Data Analytics Software), Indigobio (Bioanalytics Technology and Software), and Proniras (Pharmaceutical Research Firm). None of the other authors (AS, RB, EAE) have any conflicts of interests or biomedical interests to report.

AUTHORS' CONTRIBUTION

AS, EAE, and RAC were responsible for the fundamental design elements of the experiment, with advice from RLB. Hands on work and

data acquisition were conducted by AS and EAE. Data analysis and interpretation involved all of the authors, and primary manuscript drafting was a combined effort of AS and RAC, with EAE and RLB providing critical input on presubmission revision. All authors approved the final submitted documents.

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REFERENCES

1. Dixon L. Dual diagnosis of substance abuse in schizophrenia: prevalence and impact on outcomes. *Schizophr Res*. 1999;35:s93-s100.
2. Morisano D, Bacher I, Audrain-McGovern J, George TP. Mechanisms underlying the comorbidity of tobacco use in mental health and addictive disorders. *Can J Psychiatry*. 2009;54(6):356-367.
3. Mueser KT, Yarnold PR, Bellack AS. Diagnostic and demographic correlates of substance abuse in schizophrenia and major affective disorder. *Acta Psychiatr Scand*. 1992;85(1):48-55.
4. O'Brien CP, Charney DS, Lewis L, et al. Priority actions to improve the care of persons with co-occurring substance abuse and other mental disorders: a call to action. *Biol Psychiatry*. 2004;56(10):703-713.
5. Regier DA, Farmer ME, Rae DS, et al. Comorbidity of mental disorders with alcohol and other drug abuse. Results from the epidemiologic catchment area (ECA) study. *JAMA*. 1990;264(19):2511-2518.
6. Barnett JH, Werners U, Secher SM, et al. Substance use in a population-based clinic sample of people with first-episode psychosis. *Br J Psychiatry*. 2007;190(06):515-520.
7. Lambert M, Conus P, Lubman DI, et al. The impact of substance use disorders on clinical outcome in 643 patients with first-episode psychosis. *Acta Psychiatr Scand*. 2005;112(2):141-148.
8. Sajid A, Whiteman A, Bell RL, Greene MS, Engleman EA, Chambers R. Prescription drug monitoring program data tracking of opioid addiction treatment outcomes in integrated dual diagnosis care involving injectable naltrexone. *Am J Addict*. 2016;25(7):557-564.
9. Balhara YP, Lev-Ran S, Martinez-Raga J, et al. State of training, clinical services, and research on dual disorders across France, India, Israel, and Spain. *J Dual Diagn*. 2016;12(3-4):252-260.
10. Chambers RA, Connor MC, Boggs CJ, Parker GF. The dual diagnosis physician-infrastructure assessment tool: examining physician attributes and dual diagnosis capacity. *Psychiatr Serv*. 2010a;61(2):184-188.
11. Schmidt LM, Hesse M, Lykke J. The impact of substance use disorders on the course of schizophrenia--a 15-year follow-up study: dual diagnosis over 15 years. *Schizophr Res*. 2011;130(1-3):228-233.
12. Chambers RA. *The 2 × 4 model: a neuroscience-based blueprint for the modern integrated addiction and mental health treatment system*. First ed. New York, NY: Routledge; 2018.
13. Chambers RA, Krystal JH, Self DW. A neurobiological basis for substance abuse comorbidity in schizophrenia. *Biol Psychiatry*. 2001;50(2):71-83.
14. Lipska BK, Jaskiw GE, Weinberger DR. Postpubertal emergence of hyperresponsiveness to stress and to amphetamine after neonatal excitotoxic hippocampal damage: a potential animal model of schizophrenia. *Neuropsychopharmacology*. 1993;9(1):67-75.
15. DiFranza JR, Guerrera MP. Alcoholism and smoking. *J Stud Alcohol*. 1990;51(2):130-135.
16. Falk DE, Yi HY, Hiller-Sturmhofel S. An epidemiologic analysis of co-occurring alcohol and tobacco use and disorders: findings from the National Epidemiologic Survey on alcohol and related conditions. *Alcohol Res Health*. 2006;29(3):162-171.
17. Kandel D, Chen K, Warner LA, Kessler RC, Grant B. Prevalence and demographic correlates of symptoms of last year dependence on alcohol, nicotine, marijuana and cocaine in the US population. *Drug Alcohol Depend*. 1997;44(1):11-29.
18. Lasser K, Boyd JW, Woolhandler S, Himmelstein DU, McCormick D, Bor DH. Smoking and mental illness: a population-based prevalence study. *JAMA*. 2000;284(20):2606-2610.
19. Tseng KY, Chambers RA, Lipska BK. The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. *Behav Brain Res*. 2009;204(2):295-305.
20. Chambers RA, McClintick JN, Sentir AM, et al. Cortical-striatal gene expression in neonatal hippocampal lesion (NVHL)-amplified cocaine sensitization. *Genes Brain Behav*. 2013;12(5):564-575.
21. Chambers RA, Sentir AM, Conroy SK, Truitt WA, Shekhar A. Cortical-striatal integration of cocaine history and prefrontal dysfunction in animal modeling of dual diagnosis. *Biol Psychiatry*. 2010b;67(8):788-792.
22. Chambers RA, Sentir AM, Engleman EA. Ventral and dorsal striatal dopamine efflux and behavior in rats with simple vs. co-morbid histories of cocaine sensitization and neonatal ventral hippocampal lesions. *Psychopharmacology (Berl)*. 2010c;212(1):73-83.
23. O'Donnell P. Cortical disinhibition in the neonatal ventral hippocampal lesion model of schizophrenia: new vistas on possible therapeutic approaches. *Pharmacol Ther*. 2012;133(1):19-25.
24. Tseng KY, Lewis BL, Lipska BK, O'Donnell P. Post-pubertal disruption of medial prefrontal cortical dopamine-glutamate interactions in a developmental animal model of schizophrenia. *Biol Psychiatry*. 2007;62(7):730-738.
25. Chambers RA, Jones RM, Brown S, Taylor JR. Natural reward-related learning in rats with neonatal ventral hippocampal lesions and prior cocaine exposure. *Psychopharmacology (Berl)*. 2005;179(2):470-478.
26. Placek K, Dippel WC, Jones S, Brady AM. Impairments in set-shifting but not reversal learning in the neonatal ventral hippocampal lesion model of schizophrenia: further evidence for medial prefrontal deficits. *Behav Brain Res*. 2013;256:405-413.
27. Rao KN, Sentir AM, Engleman EA, et al. Toward early estimation and treatment of addiction vulnerability: radial arm maze and N-acetyl cysteine before cocaine sensitization or nicotine self-administration in neonatal ventral hippocampal lesion rats. *Psychopharmacology (Berl)*. 2016;233(23-24):3933-3945.
28. Berg SA, Chambers RA. Accentuated behavioral sensitization to nicotine in the neonatal ventral hippocampal lesion model of schizophrenia. *Neuropharmacology*. 2008;54(8):1201-1207.
29. Chambers RA, Taylor JR. Animal modeling dual diagnosis schizophrenia: sensitization to cocaine in rats with neonatal ventral hippocampal lesions. *Biol Psychiatry*. 2004;56(5):308-316.
30. Conroy SK, Rodd Z, Chambers RA. Ethanol sensitization in a neurodevelopmental lesion model of schizophrenia in rats. *Pharmacol Biochem Behav*. 2007;86(2):386-394.
31. Berg SA, Czachowski CL, Chambers RA. Alcohol seeking and consumption in the NVHL neurodevelopmental rat model of schizophrenia. *Behav Brain Res*. 2011;218(2):346-349.
32. Berg SA, Sentir AM, Cooley BS, Engleman EA, Chambers RA. Nicotine is more addictive, not more cognitively therapeutic in a neurodevelopmental model of schizophrenia produced by neonatal ventral hippocampal lesions. *Addict Biol*. 2014;19(6):1020-1031.
33. Chambers RA, Self DW. Motivational responses to natural and drug rewards in rats with neonatal ventral hippocampal lesions: an animal model of dual diagnosis schizophrenia. *Neuropsychopharmacology*. 2002;27(6):889-905.
34. Jeanblanc J, Balguerie K, Coune F, Legastelois R, Jeanblanc V, Naassila M. Light alcohol intake during adolescence induces alcohol addiction in a neurodevelopmental model of schizophrenia. *Addict Biol*. 2015;20(3):490-499.
35. Karlsson RM, Kircher DM, Shaham Y, O'Donnell P. Exaggerated cue-induced reinstatement of cocaine seeking but not incubation of cocaine craving in a developmental rat model of schizophrenia. *Psychopharmacology (Berl)*. 2013;226(1):45-51.

36. Khokhar JY, Todd TP. Behavioral predictors of alcohol drinking in a neurodevelopmental rat model of schizophrenia and co-occurring alcohol use disorder. *Schizophr Res*. 2018;194:91-97.
37. Brady AM, McCallum SE, Glick SD, O'Donnell P. Enhanced methamphetamine self-administration in a neurodevelopmental rat model of schizophrenia. *Psychopharmacology (Berl)*. 2008;200(2):205-215.
38. Chambers RA, Taylor JR, Potenza MN. Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. *Am J Psychiatry*. 2003;160(6):1041-1052.
39. Chambers RA, Lipska BK. A method to the madness: producing the neonatal ventral hippocampal lesion rat model of schizophrenia. In: O'Donnell P, ed. *Animal Models of Schizophrenia and Related Disorders*. Totowa, N.J: Humana Press; 2011.
40. Matta SG, Balfour DJ, Benowitz NL, et al. Guidelines on nicotine dose selection for in vivo research. *Psychopharmacology (Berl)*. 2007;190(3):269-319.
41. Czachowski CL, Samson HH, Denning CE. Blood ethanol concentrations in rats drinking sucrose/ethanol solutions. *Alcohol Clin Exp Res*. 1999;23(8):1331-1335.
42. Le AD, Lo S, Harding S, Juzytsch W, Marinelli PW, Funk D. Coadministration of intravenous nicotine and oral alcohol in rats. *Psychopharmacology (Berl)*. 2010;208(3):475-486.
43. Le AD, Funk D, Lo S, Coen K. Operant self-administration of alcohol and nicotine in a preclinical model of co-abuse. *Psychopharmacology (Berl)*. 2014;231(20):4019-4029.
44. Nestler EJ. Is there a common molecular pathway for addiction? *Nat Neurosci*. 2005;8(11):1445-1449.
45. D'Souza DC, Gil RB, Madonick S, et al. Enhanced sensitivity to the euphoric effects of alcohol in schizophrenia. *Neuropsychopharmacology*. 2006;31(12):2767-2775.
46. Caballero A, Tseng KY. GABAergic function as a limiting factor for prefrontal maturation during adolescence. *Trends Neurosci*. 2016;39(7):441-448.
47. Lew SE, Tseng KY. Dopamine modulation of GABAergic function enables network stability and input selectivity for sustaining working memory in a computational model of the prefrontal cortex. *Neuropsychopharmacology*. 2014;39(13):3067-3076.
48. Tseng KY, Lewis BL, Hashimoto T, et al. A neonatal ventral hippocampal lesion causes functional deficits in adult prefrontal cortical interneurons. *J Neurosci*. 2008;28(48):12691-12699.
49. Vohs JL, Chambers RA, O'Donnell BF, Krishnan GP, Morzorati SL. Auditory steady state responses in a schizophrenia rat model probed by excitatory/inhibitory receptor manipulation. *Int J Psychophysiol*. 2012;86(2):136-142.

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