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Nicotine is more addictive, not more cognitively therapeutic in a neurodevelopmental model of schizophrenia produced by neonatal ventral hippocampal lesions

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Abstract

Nicotine dependence is the leading cause of death in the U.S. However, research on high rates of nicotine use in mental illness has primarily explained this comorbidity as reflecting nicotine's therapeutic benefits, especially for cognitive symptoms, equating smoking with 'self-medication'. We used a leading neurodevelopmental model of mental illness in rats to prospectively test the alternative possibility that nicotine dependence pervades mental illness because nicotine is simply more addictive in mentally ill brains that involve developmental hippocampal dysfunction. Neonatal ventral hippocampal lesions (NVHL) have previously been demonstrated to produce post-adolescent-onset, pharmacological, neurobiological and cognitive-deficit features of schizophrenia. Here, we show that NVHLs increase adult nicotine self-administration, potentiating acquisition-intake, total nicotine consumed, and drug-seeking. Behavioral sensitization to nicotine in adolescence prior to self-administration is not accentuated by NVHLs in contrast to increased nicotine self-administration and behavioral sensitization documented in adult NVHL rats, suggesting peri-adolescent neurodevelopmental onset of nicotine addiction vulnerability in the NVHL model. Delivering a nicotine regimen approximating the exposure used in the sensitization and self-administration experiments (i.e. as a treatment) to adult rats did not specifically reverse NVHL-induced cortical-hippocampal-dependent cognitive deficits and actually worsened cognitive efficiency after nicotine treatment stopped, generating deficits that resemble those due to NVHLs. These findings represent the first prospective evidence demonstrating a causal link between disease processes in schizophrenia and nicotine addiction. Developmental cortical-temporal limbic dysfunction in mental illness may thus amplify nicotine's reinforcing effects and addiction risk and severity, even while producing cognitive deficits that are not specifically or substantially reversible with nicotine.

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Authors Contribution

SAB and RAC were involved in all components of the experimental design, execution, data analysis and manuscript preparation. AMS and BSC played critical roles in the management of rats through the longitudinal experiment and the hands on data collection involving self-administration and RAM testing. RAC and EAE co-supervised the project and were integral to manuscript preparation.

Keywords

nicotine dependence; dual diagnosis; self-administration; schizophrenia; addiction; hippocampus

Introduction

With pathogenic effects spanning brain and cardio-pulmonary systems, nicotine dependence remains the single largest cause of death in the U.S. (Mokdad et al., 2004). As general population rates have fallen below 25%, smoking has become more concentrated in the mentally ill who now consume around half of all cigarettes sold (Grant et al., 2004; Lasser et al., 2000). Smoking rates exceeding 75% in schizophrenia populations are associated with decades cut from individual lifespans, lower psychiatric treatment compliance, and financial impoverishment as government assistance for the mentally ill is channeled into tobacco industry profits (Parks et al., 2006; Prochaska et al., 2008; Steinberg et al., 2004).

Psychiatric research on nicotine use in mental illness has traditionally been guided by the hypothesis that this comorbidity reflects therapeutic effects of nicotine and/or tobacco, so that smoking in mental illness is widely accepted as synonymous with ‘self-medication’ (Dani and Harris, 2005; DeHay et al., 2012). Human data encompassing genetic and histological analyses of nicotinic receptors and electrophysiological and cognitive responses to nicotine have been suggested to reflect schizophrenia-specific abnormalities that allow nicotine to function as a cognitive enhancer in this illness (Dani and Harris, 2005; Leonard et al., 2001). Although this research has imparted neuroscientific credence to a medicinal value for nicotine use in schizophrenia, recently emerging data indicate that acute nicotine dosing has no cognitive therapeutic benefits for schizophrenic compared to non-schizophrenia smokers (Hahn, in press). Also, growing evidence suggests that chronic nicotine exposure may actually cause rather than treat cognitive and affective symptoms (Counotte et al., 2011; McDermott et al., 2013; Reitz et al., 2007; Slotkin, 2008).

Generally, the self-medication hypothesis has not translated well into motivating clinicians or patients to treat nicotine addiction (DeHay et al., 2012), nor does it effectively explain why schizophrenia patients have increased addictions to other drugs like cocaine and alcohol, which are known for exacerbating rather than improving psychotic and/or cognitive symptoms (Volkow, 2009). Given this larger picture, an alternative hypothesis becomes apparent: the connection between schizophrenia and nicotine dependence may reflect a more general, and involuntary biological process where one brain disease (i.e. schizophrenia) predisposes to and synergizes with another (i.e. addiction) (Chambers et al., 2001).

Directly testing this alternative hypothesis necessitates pre-clinical approaches, not ethically possible in human subjects, where addictive drugs can be prospectively tested in wellcontrolled experiments using heuristic, drug-naive animal models of mental illness. For this purpose, we have applied the neonatal ventral hippocampal lesion (NVHL) model of schizophrenia. In this model, the axon-sparing neurotoxin ibotenic acid is delivered into the hippocampus of 7-day-old rats corresponding to the second trimester human fetal-brain development when environmental and genetic risk factors are implicated in seeding schizophrenia (Lipska et al., 1993; Weinberger, 1999). Similar to observations in humans

with schizophrenia, NVHL rats have hippocampal atrophy that is proportional to overall syndrome severity (Brambilla et al., 2013; Chambers et al., 1996), and many secondary neurobiological and behavioral abnormalities involving prefrontal-cortical-striatal anatomy and function (Tseng et al., 2009). Developmentally, NVHLs produce post-adolescent onset of 'positive' symptom-like behavioral abnormalities that are reducible with antipsychotic medications, superimposed on more insidiously presenting, earlier onset, cognitive and 'negative' symptoms that do not respond to anti-psychotics (Tseng et al., 2009).

Illustrative of a fundamental neurobiological connection between severe mental illness and addiction vulnerability, NVHLs also cause an involuntary amplification of short- and long-term behavioral sensitization to cocaine (Chambers and Taylor, 2004), alcohol (Conroy et al., 2007) and nicotine (Berg and Chambers, 2008), corresponding to increased self-administration of cocaine (Chambers and Self, 2002) and alcohol (Berg et al., 2011). The present study was designed to test whether this addiction vulnerability generalizes to nicotine, and to capture first proof of a causative relationship between early disruptions of hippocampal network development and adult-age nicotine addiction vulnerability, co-occurring with cognitive impairments that may or may not respond therapeutically to nicotine.

Methods and Materials

Subjects and Neonatal Surgeries—Subjects were born in our facility from Sprague-Dawley females arriving at 14–16 days gestation (Harlan, Indianapolis, IN). Post-natal Day (PD)-5 litters were culled to males in preparation for surgeries on PD-7. Pups weighing 16–19 grams underwent surgeries performed under hypothermic anesthesia. Briefly, as described elsewhere (Lipska et al., 1993), stereotaxic-assisted Hamilton needle placement into the ventral hippocampus bilaterally (AP −3.0 mm, ML ± 3.5 mm, and DV-5.0 mm from bregma) was followed by Ibotenic acid (3.0 μg; Sigma, St. Louis, MO) delivery in 0.3 μl artificial cerebrospinal fluid (aCSF) to NVHL rats, or aCSF only to Sham-operated rats. Pups were returned to their mothers awake after 30 minutes of recovery on a heating pad, and thereafter reared under standard conditions until weaning on PD 21. At weaning, NVHL and Sham rats were housed in pairs (like lesion status) until adulthood (PD56), when they were individually housed. Surgical and experimental procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and the Indiana University Institutional Animal Care and Use Committee.

Nicotine preparation—Nicotine hydrogen tartrate salt (Sigma, St. Louis, MO) was dissolved in 0.9% sterile saline to a stock solution dose of 0.5 mg/ml (expressed in terms of the base of the salt (Matta et al., 2007)), adjusted to a pH 7.4. This solution was injected sc for adolescent sensitization and in the cognitive testing as pre-injections in volumes of 1 ml per kg of rat weight. For iv self-administration, doses were prepared daily on a per rat weight basis from stock solutions.

Adolescent Behavioral Sensitization to Nicotine—During mid-adolescence (PD-34 to 44), rats destined for adult nicotine self-administration were given 10 once daily injections (sc) of nicotine (0.5 mg/kg) or saline (1ml/kg) during locomotor testing in 43 × 43

cm plexiglass arenas (Med Associates, St. Albans, VT), equipped with 16 infra-red beam arrays. Position, track, distance, speed and non-ambulatory movements were recorded over 2 hour sessions. Rats were tested under red light and had injections delivered at the beginning of the second hour.

Adult IV Cannulation and Nicotine Self-administration—On PD-56, subjects entering self-administration underwent jugular venous catheterization under sodium pentobarbital anesthesia. As detailed elsewhere (Chambers and Self, 2002), Silastic tubing (Green Rubber, Worburn, MA) placed into the animal's right vein coursed subcutaneously over the shoulder to exit the back via a 22-gauge cannula (Plastics One, Roanoke VA). Catheters were flushed daily with 0.3 ml heparinized saline (20 IU/ml) containing gentamycin sulfate (0.13mg/ml). Catheter patency checks were conducted once a week on weekends using a 1mg/0.1 ml iv push of methohexital sodium (Henry Schein, Indianapolis, IN), which produces a 10–20 second loss of consciousness with patent catheters. Rats with failed or infected catheters were excluded from the experiment.

In preparation for nicotine self-administration on PD-59, to promote exploratory behavior, food restriction was started, maintaining body weight at 85% of pre-restriction weight (with 2–3 bricks/day of standard rat chow). This restriction continued for all rats until the 5th acquisition session. Self-administration sessions began on PD-60 in Med Associates chambers controlled by software that recorded instrumental activity. These units, housed inside sound-attenuating cubicles, were equipped with 2 non-retractable levers with cue lights, a house light, and an infusion pump assembly (Razel Model A pump; Med Associates).

All self-administration sessions were 2 hours long beginning with house light on to signal nicotine availability and a single priming infusion of nicotine. Responses on the active lever (counterbalanced left/right between animals) resulted in house lights off and drug-paired lever cue light on for a 3 second infusion (0.015 mg/kg nicotine in 0.050 ml saline; FR1 schedule). A 17-second time out followed with all lights out during which recorded lever presses produced no consequences. Rats progressed through 3 stages of sessions: acquisition, dose response testing and extinction, with no days off between stages. For acquisition, rats were given a maximum of 35 once daily sessions, during which they were regarded as having acquired self-administration when they had accumulated (not necessarily consecutively) 20 days of > 20 active lever presses (nicotine hits) per day (i.e. resulting in a minimum exposure of 6 mg/kg nicotine during acquisition). These sessions occurred on a Monday-Friday (5 day) schedule with weekends (2 days) off so that rats remained in acquisition from 4 to 7 weeks depending on performance. Through acquisition, any rat that did not press once on the active lever in the prior session had a single sucrose pellet placed on the active lever for the subsequent session. At the end of acquisition upon reaching the 20 days/20 hits criteria or the 35^{th} session, whichever came first, rats progressed on the very next day to the dose response stage during which they had access to 7.5, 15, 30 and 15 μg nicotine/kg/infusion (one dose per session; not counter-balanced) over 4 daily sessions. The day after their last dose response day, rats began once daily extinction sessions (2 hours; no nicotine pre-injections; house lights only on; lever presses producing no consequences),

proceeding through these sessions until responding with ≤ 5 presses on the previously active lever and ≤ 10 presses on the inactive lever.

Adult Radial Arm Maze Testing—Rats separate from those undergoing self-administration were prepared for cognitive testing on the Radial Arm Maze (RAM) beginning on PD-60, with food restriction (to maintain weight at 85% of pre-restriction weight) starting on PD-59. Rats were fed regular chow after daily sessions. The RAM (Med Associates) was constructed in plexiglass and equipped with a central octagonal arena (29.5 cm diameter) with 8 runways extending radially (61 × 9 cm with 17 cm high walls), standing 6.5 cm above the floor and surrounded by consistent visual landmarks.

In spatial learning and working memory testing based on the win-shift paradigm (Olton and Samuelson, 1976), rats learned in daily sessions to efficiently enter all 8 arms of the maze as reinforced by ½ of a Kellogs® Froot Loop® loaded at the end of each arm. Sessions lasted a maximum 300 seconds, or when animals had entered all 8 arms, whichever came first. Three primary dependent measures were 1) entries-to-repeat (ETR: the total number of arms entered before the rat repeated an arm entry; 2) total session time and 3) Froot Loops consumed. Rats were tested over 24 (once daily) sessions organized into 8 blocks of 3 sessions, spanning 5 sequential phases. In phase 1 (block 1), all animals received a pre-injection of saline (sc) 30-minutes prior to the session. In phase 2 (blocks 2–4), NVHL and Sham rats were randomized to receive saline or nicotine injections (0.5 mg/kg sc) 30-minutes prior. In phase 3, (blocks 5 and 6) all animals were only given saline pre-injections. Subsequently animals were given a 2 week break in their home cages, then resumed testing for Phase 4 (block 7) in which they were all given saline pre-injections followed by Phase 5 (block 8) when they were all given nicotine (0.5 mg /kg) pre-injections.

Histology—After behavioral testing, rats were sacrificed by decapitation under brief isoflurane anesthesia. Brains were removed whole and cryostat cut into coronal sections (40 μm) through the rostral-caudal extent of the hippocampus. Mounted sections were fixed and thionin (0.5%) stained. Microscopic examination of sections for lesion verification was performed separate from, and blind to behavioral data; rats without appropriate lesions were excluded from the study. Appropriate lesions were identified as those showing bilateral evidence of tissue atrophy, paucity of nuclei, and cellular disarray (with lateral ventricular enlargement) confined to the ventral hippocampus (Fig 1). Brains with unilateral damage, damage encompassing the dorsal blades of the hippocampus, or direct damage to nearby structures (temporal cortex, amygdala, thalamus, basal ganglia) were excluded. From the self-administration experiment, 23 of 33 rats (70%) that underwent ibotenic acid delivery and had successful catheters, had appropriate hippocampal malformations and were included in the study. From the RAM experiment, 17 of 25 (68%) of ibotenic-exposed rats had acceptable NVHLs.

Data Analysis—Parametric testing examined dependent variables of locomotor activity (cm/hr) in behavioral sensitization. Active and inactive lever pressing, nicotine intake, and time-out pressing (on active and inactive levers) were examined in self-administration. ETR, session time and Froot Loops consumed, taken as the mean of 3 consecutive sessions for each block were dependent measures in RAM testing. Two-way Analysis of Variance

(ANOVA) with independent variables lesion status and nicotine history were used with repeated measures testing across multiple sessions as appropriate. In sensitization/self-administration, the nicotine variable referred to adolescent nicotine vs. saline exposure. In RAM testing, the nicotine variable reflected nicotine vs. saline pre-injections during maze learning. Separate ANOVAs were applied to different phases of self-administration and RAM experiments. Significance was identified at $p<0.05$ with mention of notable negative or marginal effects. Wherever significant interactions occurred between main effects and repeated time measures, secondary one-way ANOVAs were applied to specify when in the repeated measure the main effect was strongest.

Results

Adolescent nicotine sensitization and adult self-administration—In the first experiment, 45 rats first underwent daily experimenter-delivered injections of nicotine (0.5 mg/kg sc) (NVHL, n=12; Sham, n=10) or saline (1ml/kg sc) (NVHL, n=11; Sham, n=12) for 10 days during mid-adolescence (PD 35- 44) followed by adult self-administration (PD-60). This early nicotine exposure tested whether abnormal nicotine responsiveness occurs in NVHLs prior to the peri-adolescent onset of the full syndrome, and whether it interacts with NVHLs to alter adult self-administration. Although adolescent nicotine injections robustly sensitized locomotor activity (day × nicotine: $F_{9, 369}=39.8$, $P<0.001$; nicotine: $F_{1,41}=261.5$, $P<0.001$), adolescent NVHL rats did not sensitize differently from Shams (Fig. 2).

Upon reaching adulthood (PD-56), these 45 rats underwent jugular venous catheterization followed by iv nicotine self-administration 4 days later. Only the first 20 days of acquisition were analyzed (Fig. 3a–d) since on acquisition days 21–35, treatment group n's began to drop differentially as rats met acquisition criteria (20 days >20 nicotine infusions/day) and progressed to dose response testing. Over the first 20 days, nicotine was generally self-administered in increasing amounts via active lever presses (i.e. presses that delivered infusions) (days: $F_{19,779}=14.6$, $P<0.001$) (Fig. 3a), with no growth in inactive lever pressing (Fig 3b). NVHL rats showed stronger acquisition in terms of active lever pressing (lesion: $F_{1,41}=16.0$, $p<0.001$) and shape of the acquisition curve (lesion × days: $F_{19,779}=2.05$, $P<0.01$). Post-hoc ANOVAs (one way by lesion status on each day) detected significant increases in NVHL responding initially emerging on days 4 and 5, then becoming larger and more frequent over the next 3 weeks of acquisition. NVHLs did not differ from Shams on inactive lever pressing but they did show increased active lever time-out responding (lesion: $F_{1,41}=9.1$, $P<0.01$) (Fig. 3c). Time-out responding at the inactive level was flat and not different between groups (Fig. 3d).

Based on the acquisition criteria in which all rats were given up to 35 days to achieve 20 days of ≥20 nicotine infusions/day, NVHL rats achieved acquisition criteria in fewer days than Shams (lesion: $F_{1,41}=7.3$, $P<0.01$) (Fig. 3e) and therefore entered the dose response testing earlier. This provided the Shams with the opportunity of more acquisition sessions to catch up to the NVHLs in terms of cumulative nicotine intake. Even with this experimental design, NVHLs still had a greater total nicotine intake (lesion: $F_{1,41}=9.0$, $P<0.01$) (Fig. 3f) as calculated over all acquisition sessions.

Adolescent nicotine exposure had no effects or interactions on any measure during the acquisition stage of nicotine self-administration. However, during dose response testing analyzed over 5 days, in which the last acquisition session was considered the first dose response session, a dose-dependent effect of adolescent nicotine history on nicotine intake did emerge without lesion effects (day × nicotine history: $F_{4,164}$=3.4, P<0.05) (Fig. 4a). One way (by nicotine history) post-hoc ANOVAs performed across dosing days detected a nicotine-history associated increase in nicotine intake at the 30 μg dose ($F_{1,44}$=4.1, P<0.05). As expected for dose response testing, both daily nicotine intake (Fig. 4a) and active lever presses (Fig. 4b) varied significantly across days ((day: $F_{4,164}$=40.3, P<0.001) and (day: $F_{4,164}$ =10.1, P<0.001) respectively). However, inactive lever pressing did not differ across days or by lesion status or nicotine history. In parallel to active lever pressing, time-out active lever pressing did vary significantly across days (day: $F_{4,164}$ =9.2, P<0.001) (Fig. 4d), also without lesion or nicotine history effects. Time-out inactive lever pressing (Fig. 4e) also varied across days (day: $F_{4,164}$ =3.1, P<0.05) with a day × lesion interaction ($F_{4,164}$ =2.5, P<0.05), in which Shams pressed more on the middle day (15 μg dose) ($F_{1,44}$=5.7, P<0.05). Despite this statistical significance, these effects in time-out inactive lever pressing were likely not meaningful due to the extremely low responding (averaging <3 hits per 2 hours) observed over the course of dose response testing.

Having established that total nicotine intake and active lever presses did not differ over the 5 dose response sessions according to lesion status, we also determined more specifically that NVHL and Sham rats also did not differ on active or inactive lever pressing on their very last day of nicotine intake (dose response day 5; 15 μg dose). This confirmed that despite robust lesion-based differences in active lever responding and nicotine intake over the acquisition stage, the added acquisition sessions allotted to Sham rats did allow NVHL and Sham rats to arrive at comparable levels of nicotine reinforcement and exposure by the time of (and measured over) the dose response days just before extinction testing. Thus, lesion-based differences in subsequent extinction responding can be interpreted as signifying persistent changes in nicotine-seeking behavior due to NVHLs, independent from possible effects of very recent drug-taking behavior, although lesion-based differences in nicotine intake earlier in acquisition might be still be predictive of, or contribute to, later extinction differences. Indeed, in the first extinction session when rats pursued nicotine in daily 2 hour sessions, but without nicotine reinforcement, NVHL rats again demonstrated greater active lever responses (lesion: $F_{1,41}$=7.5, P<0.01) (Fig. 5a). When dividing the first extinction session into 4 × 30 minute segments, this main effect was accompanied by a significant within-session tapering of active lever pressing from an initial extinction burst (segment: $F_{3,123}$=94, P<0.001). Compared to their overall average of 8.1 presses per 30 minutes recorded in their final dose response session when they were receiving nicotine, active lever pressing was higher for all rat groups in the first 30 minutes of the first extinction session, dropping below the 8.1 average across the 30 to 120 minute segments. A lesion × segment interaction ($F_{3,123}$=8.2, P<0.001) indicated that NVHLs amplified the magnitude of the extinction burst as confirmed by a post-hoc one-way (lesion status) ANOVA testing at each time segment, which showed a significant increase for NVHLs in the first 30 minute segment ($F_{1,44}$=8.2, P<0.001). In analysis of extinction responding over a longer time span encompassing the first 3 days of extinction testing, NVHL rats also showed persistent

elevations in drug-seeking on the previously nicotine paired lever (lesion: $F_{1,36}$=8.6, P<0.01), superimposed on an extinguishing pattern of responding across days (day: $F_{2, 72}$=30.2, P<0.001) (Fig. 5b). On the inactive lever, where responding was still much less overall than at the previously nicotine-paired lever, an overall extinction pattern was also observed (day: $F_{2, 72}$=8.7, P<0.001) with Sham rats tending to have higher responding than NVHLs on day one that habituated significantly more compared to NVHLs over the 3 days (day × lesion: $F_{2,72}$=5.5, P<0.01) (Fig 5c).

Adult learning and working memory deficits and response to nicotine— Different sets of NVHL and Sham rats (n=37) entered a second experiment beginning in adulthood (PD- 60) that tested the effects of nicotine on learning and working memory performance on the 8-arm radial-arm maze (RAM). This testing measures prefrontal-cortical-hippocampal network dysfunction analogous to that underlying the contextual-spatial working memory deficits in human schizophrenia (Fuller et al., 2009; Gold et al., 2010) and is sensitive to nicotinic receptor manipulation and the NVHL model (Chambers et al., 1996; (Levin, 1988). All rats received saline pre-injections 30 minutes before testing across blocks 1, 5, 6, and 7 but were randomized to receive 9 (once daily) nicotine pre-injections (0.5 mg/kg sc) (NVHL, n=9; Sham, N=10) or saline (NVHL, n=8; Sham, n=10) before testing across blocks 2–4. By design, this dosing regimen closely approximated that used in the first experiment. In nicotine sensitization, the 10 × 0.5 mg/kg doses (5 mg/kg total exposure) were behaviorally activating (compared to saline) from 10 to 60 minutes post-injection. In nicotine self-administration, the acquisition criteria of a minimum of 20 infusions/day × 20 days would produce a total exposure of approximately 6 mg/kg within 20 to 35 sessions.

Over the first block of RAM testing, NVHL rats showed no impairments in entries-to-repeat (ETR) (Fig. 6a) but were significantly slower in session time (lesion: $F_{1,33}$=2.1, P<0.05) (Fig. 6b) while showing no differences in Froot Loops eaten (Fig 6c). Across blocks 2–4, all rats demonstrated learning with increased ETRs (blocks: $F_{2,66}$=13.6, P<0.001), decreased session times (blocks: $F_{2,66}$=63.5, P<0.001), and more Froot Loops eaten (blocks: $F_{2,66}$=38.5, P<0.001). Now, NVHL rats did show impaired cognition with lower ETRs (lesion: $F_{1,33}$=26.7, P<0.001) that could not be due to differences in food reward motivation since there were no lesion-based differences in Froot Loops consumed. Efficiency in completing the maze was also again impaired by NVHLs (lesion: $F_{1,33}$=4.6, P<0.05), but enhanced by nicotine pre-injections (nicotine: $F_{1,33}$=5.5, P<0.05). Nicotine pre-injections did not improve ETR however, and did not interact with NVHLs to specifically reverse NVHL deficits in ETR or session time.

Over blocks 5 and 6, when all rats were again receiving saline pre-injections, the recent nicotine exposure produced new cognitive deficits in terms of marginally worsening ETR (nicotine: $F_{1,33}$= 3.6, P=0.06) and significantly worsening session time (nicotine: $F_{1,33}$=4.4, P<0.05). NVHL deficits in ETR (lesion: $F_{1,33}$=21.8, P<0.001) and session time (lesion: $F_{1,33}$=14.8, P<0.01), persisted across these blocks and were not interactive with the effects of prior nicotine exposure. Fig. 7 depicts the session data covering the transition from nicotine pre-injections back to saline pre-injections (bins 4 through 6). These groupings

plotted according to nicotine exposure (Fig. 7a) and lesion status (Fig. 7b), allow a more clear view of the effects of nicotine withdrawal (and NVHLs) on cognition.

After block 6, all animals had 2 weeks off from RAM testing so that subsequent blocks would serve as measures of long term recall. Nicotine pre-injections were given in the final (8^{th}) block to all rats to test for potential 'nicotine rescue' effects of any cognitive deficits. Across blocks 7 and 8, NVHL deficits in ETR (lesion: block #7: $F_{1,33}$=12.2, P<0.01; block #8: $F_{1,33}$ =10.6, P<0.01) (Fig. 6a) and session time (lesion: block #7: $F_{1,33}$= 7.0, P<0.05; block #8: $F_{1,33}$=11.7, P<0.01) (Fig. 6b) persisted, with no group differences in Froot Loops consumed (Fig. 6c) and no effects of prior nicotine (or nicotine history interactions with NVHLs) on any measures.

Discussion

This study demonstrates that early developmental hippocampal damage increases the reinforcing effects of nicotine in adulthood while also producing cognitive impairments that are not specifically treated by nicotine. This modeling accurately simulates clinical phenomenology of greater severities of nicotine dependence in mentally ill people, including observations of schizophrenia patients consuming more nicotine than non-schizophrenic, nicotine dependent subjects (Williams et al., 2005). While contributing to mounting evidence pointing to the importance of hippocampal function in the pathogenesis of addictive disorders (Chambers, 2012; Chambers et al., 2001; Sudai et al., 2011), these findings replicate, and begin to biologically explain, enhanced nicotine addiction vulnerability in the absence of specific cognitive therapeutic effects of nicotine in schizophrenia subjects (Hahn, in press).

NVHLs impact the maturation and function of prefrontal cortical-ventral striatal circuits to which the ventral hippocampus directly projects, in multiple ways that correspond to neural and behavioral findings in human schizophrenia and subjects with addictions (Chambers et al., 2001; Heerey et al., 2008; Liu et al., 1998; Tseng et al., 2009). Behaviorally, NVHL rats show baseline cognitive impulsivity in their approach to natural rewards that is worsened by prior cocaine history (Chambers et al., 2005), mirroring impulsivity found in populations with nicotine dependence and other addictions (Bickel et al., 1999). The present study identified impulsive and perseverative styles of nicotine seeking in the NVHL model like those previously shown in cocaine self-administration (Chambers and Self, 2002). Specifically, NVHL rats showed increased active lever time-out responding during acquisition of nicotine self-administration and increased nicotine seeking in extinction, that mirror the same abnormalities they show in cocaine self-administration. Together, these findings confirm that NVHLs produce a failure in inhibitory control over motivated behavior associated with multiple addictive drugs abused at particularly high rates in schizophrenia.

Both NVHLs and human smokers show prefrontal cortical regional atrophy (Chambers et al., 2010a; Durazzo et al., 2013). In NVHL rats, this prefrontal atrophy corresponds to pyramidal cell neuronal atrophy and derangements in excitatory and inhibitory neurotransmission, occurring on top of neo-striatal super-sensitivity to dopamine signaling (Chambers et al., 2010a;Chambers et al, 2010b; Tseng et al., 2007). Together, these

abnormalities may contribute to enhanced recruitment of striatal activation patterns associated with addictive drug-induced behavioral adaptation in NVHL rats (Chambers et al., 2010a; Tseng et al., 2007) resulting in augmented behavioral sensitization and self-administration with multiple addictive drugs (Berg et al., 2011; Chambers and Self, 2002; Chambers et al., 2010a; Conroy et al., 2007).

Given that the NVHL model increases behavioral sensitization to nicotine in adulthood (Berg and Chambers, 2008) but not in adolescence as shown here, we can surmise that heightened nicotine responsiveness due to early hippocampal perturbation is involuntary, and emerges developmentally in phase with the post-adolescent onset of the full schizophrenia syndrome of the model. These findings comport with emerging clinical and neuroimaging data suggestive of a developmental coincidence, and neurobiological connection between nicotine addiction vulnerability and schizophrenic pathology in the brain, in which altered maturation of prefrontal-cortical striatal circuits plays a major role (Chambers et al., 2003; Compton et al., 2009; Zhang et al., 2010). Abnormalities in cortical-hippocampal network architecture and function, due to a large variety of genetic and early environmental backgrounds are implicated across mental illnesses other than schizophrenia — including post-traumatic stress disorder, borderline personality and primary mood disorders (Bremner et al., 2000; Teicher et al., 2012)—which also encompass elevated rates of nicotine addiction (Grant et al., 2004; Lasser et al., 2000; Pulay et al., 2010). Accordingly, the present findings may also illustrate a more general role of hippocampal malfunction in generating addiction vulnerability leading to many ‘dual diagnosis’ combinations that involve nicotine.

Adolescent nicotine exposure in rats similar to what we employed can produce adult prefrontal cortical physiological abnormalities with cognitive deficits that resemble those characterized in schizophrenia (Counotte et al., 2011; Counotte et al., 2009). Adolescent nicotine injections also amplify nicotine sensitization tested in adulthood (Bracken et al., 2011). Our study design did not test these effects, and we did not see adolescent nicotine sensitization impact adult self-administration in the same way that the NVHL model did. The relative lack of effect of the adolescent nicotine exposure on subsequent adult nicotine self-administration may have been due to several concurrent factors: The adolescent exposure was not self-administered, it was not delivered iv, and it was delivered in a different context from the adult self-administration. Further, as hinted by the dose response testing, where only the 30 μg dose revealed a significant adolescent nicotine-history effect, the 15 μg dose we used in acquisition may have been too low to reflect prior nicotine exposure effects. Finally, it is possible that NVHLs pushed nicotine reinforcement to a ceiling where relatively weaker nicotine dose history effects were largely obscured.

In cognitive testing, adult nicotine exposure produced mild learning benefits at least in terms of time efficiency in completing the RAM. However this beneficial effect was not specific to NVHL rats, improving Sham performance as well. Nicotine also did not at all ameliorate the primary cognitive deficit measure of ETR that is actually the most robustly impaired dimension of cognition in NVHLs measured on the RAM. Finally, the non-specific mild cognitive benefit of nicotine came at a price in terms of actually impairing performance once the nicotine stopped, producing new deficits that resembled a mild form of what the NVHL

model does to cognition even without nicotine exposure. Again, this nicotine withdrawal effect occurred non-specifically in NVHL and Sham rats alike, indicating that the net short–term cost-benefits of nicotine on cognition in the NVHL are not different from Shams. These observations are consistent with rigorously-controlled human experimentation demonstrating a lack of differential cognitive benefits of nicotine in healthy vs. schizophrenia nicotine users, and detrimental cognitive effects of nicotine withdrawal (Hahn, in press). Together with emerging animal and human data suggesting that chronic nicotine exposure can actually worsen cognition and other psychiatric symptoms (Counotte et al., 2011; McDermott et al., 2013; Reitz et al., 2007; Slotkin, 2008), our findings suggest the feasibility of two, non-contradictory, bidirectional causal dynamics underlying the link between schizophrenia and nicotine addiction, in which each disease worsens the severity of the other. We did not test every possible manner in which nicotine could work as a medicine for abnormalities in the NVHL model or schizophrenia, and therefore cannot rule out the possibility that nicotine could still be therapeutic in some way. However, our results showing that early developmental perturbation of the hippocampus amplifies the reinforcing action of nicotine, while also producing cognitive problems that are not specifically or differentially treatable with nicotine, calls into question the self-medication hypothesis as the most widely espoused explanation for high rates of nicotine dependence in schizophrenia.

These findings highlight what could be a central pitfall in the self-medication explanation in that it focuses on, and promotes, only a therapeutic value to nicotine, while ignoring its highly addictive activity, and the likelihood that it is this activity that is pathologically amplified by the biology of mental illness. In circumventing this issue, these data provide a new view on neurodevelopmental mechanisms that predispose the mentally ill to nicotine addiction that should be studied further in this model and in human subjects for discovery of new prevention and treatment strategies. Perhaps more immediately, these findings provide a neuroscientific demonstration accessible to clinicians and patients alike that identifies nicotine dependence, not as a medication-modality for mental illness, but as a comorbid addiction to which the mentally ill are the highly biologically vulnerable.

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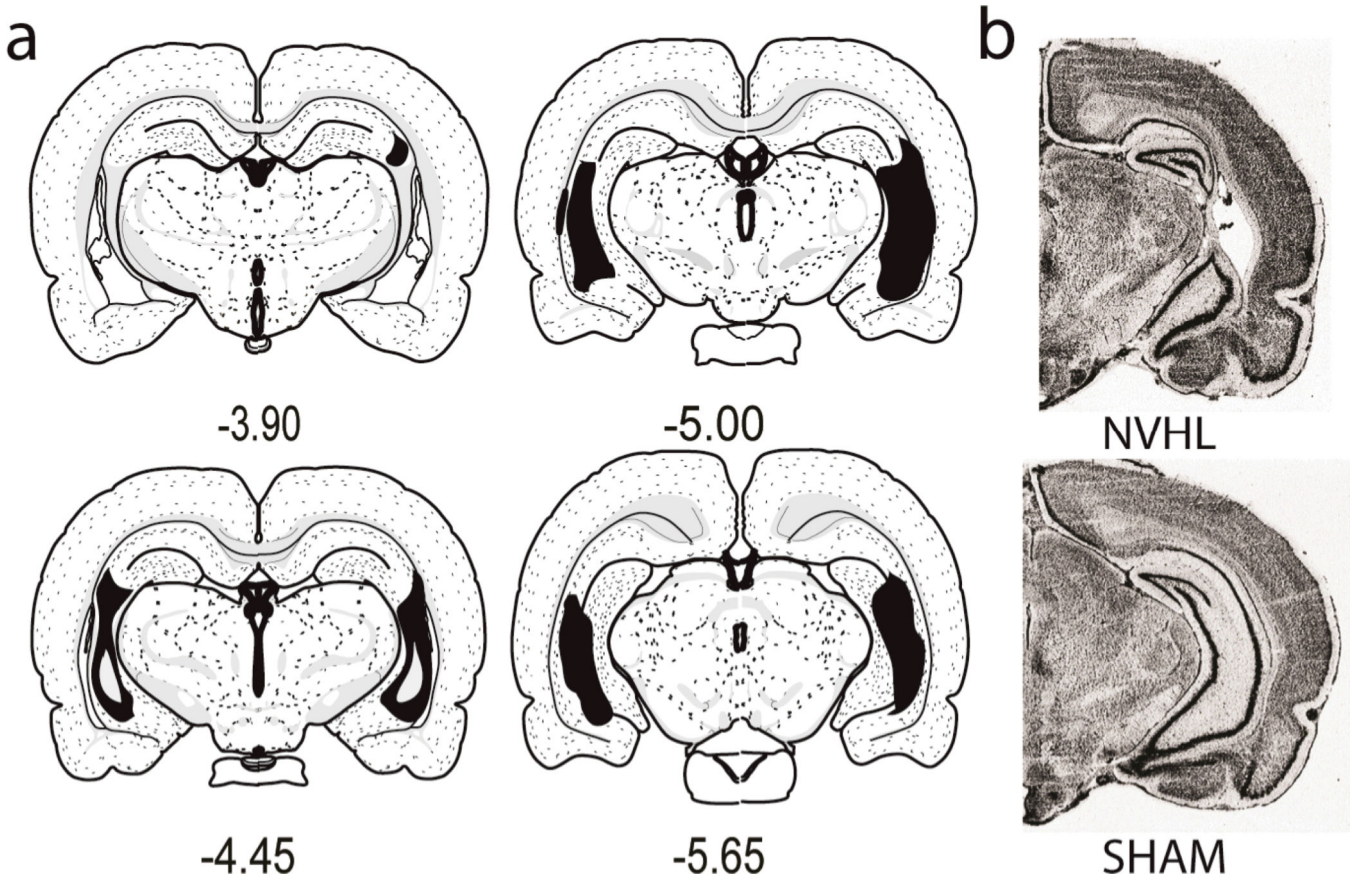

Figure 1.

Mapping of hippocampal damage in NVHL rats.(**a**) Coronal maps (from bregma (mm)) show the rostral-caudal extent of largest (black) to smallest (white inset) hippocampal damage among the 82 rats in the study. (**b**) Photomicrographs show typical NVHL histology vs. a SHAM-operated control brain. (Maps are adapted from Swanson, LW. (2004) Brain Maps: Structure of the Rat Brain. 3rd Edition, Elsevier, New York.)

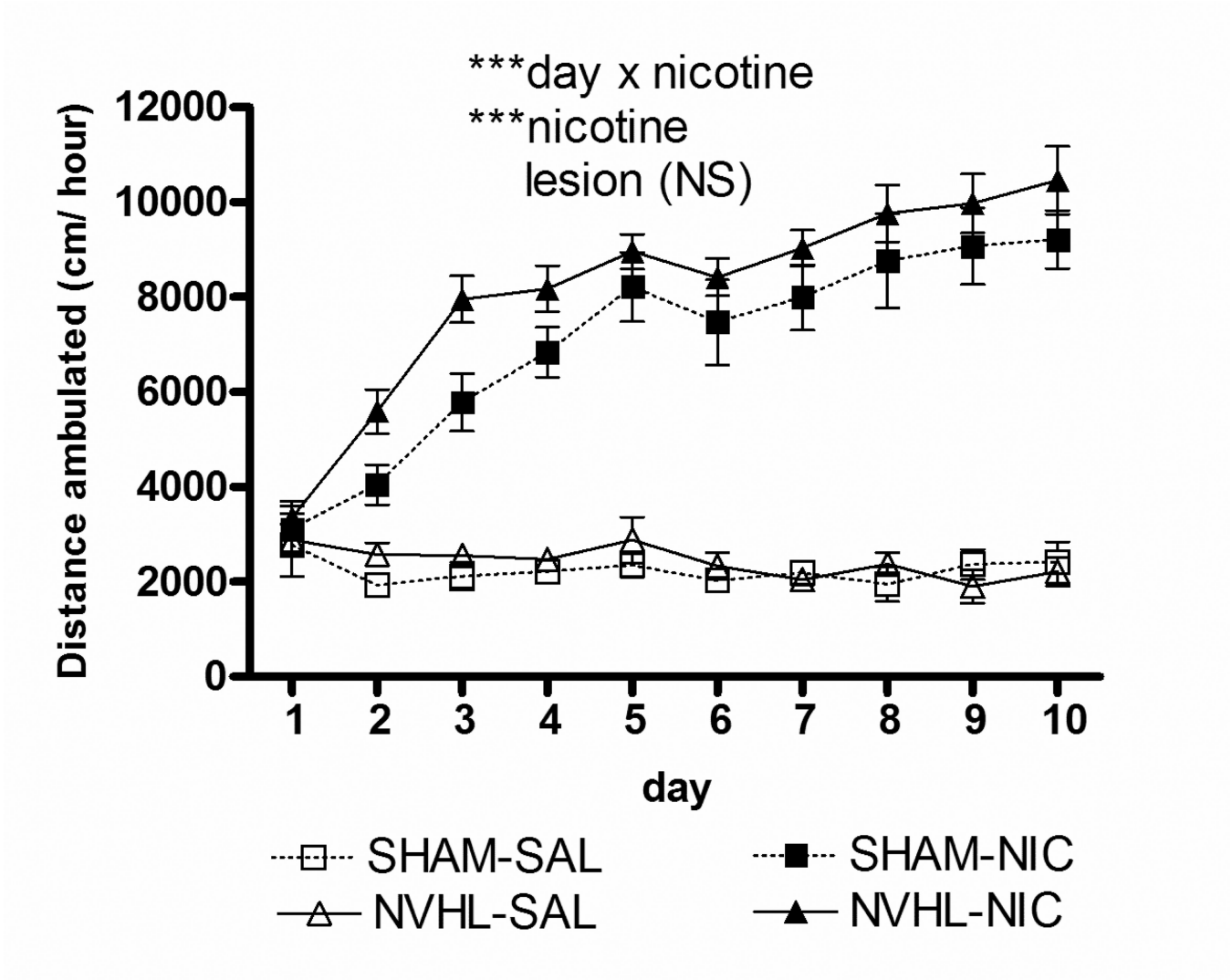

Figure 2.

Adolescent nicotine behavioral sensitization is demonstrated as growth in post-injection locomotion due to nicotine injections (***P<0.001) over 10 days. NVHL rats (nicotine (NIC) (n=12); saline (SAL) (n=11)) did not differ from SHAMS (nicotine (n=10); saline (n=12)). Data depicted as means ± SEM.

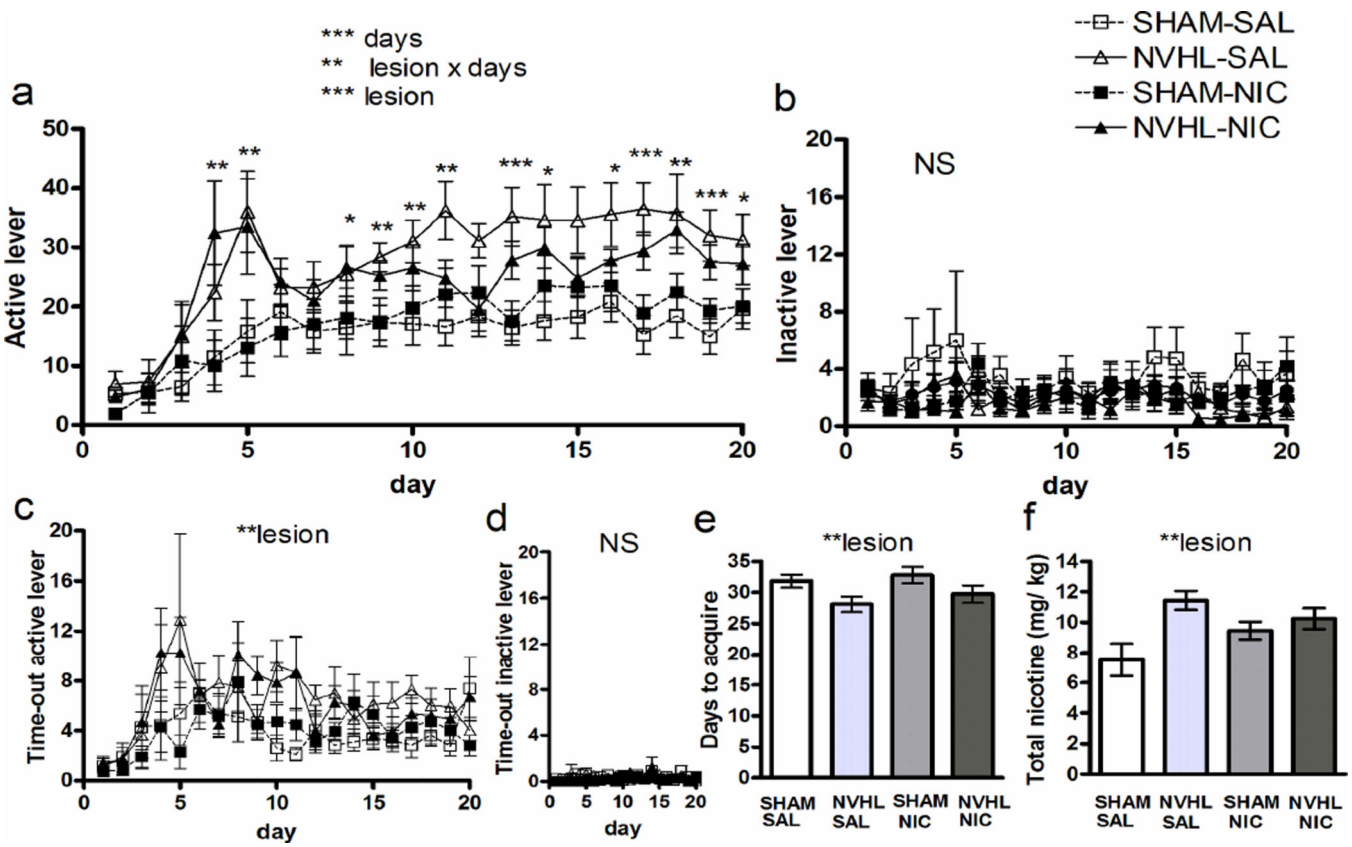

Figure 3.

Adult acquisition of nicotine self-administration, same subjects (n=45) as in Fig 2. (**a**) Over the first 20 days, nicotine was self-administered in increasing amounts via active lever presses (***days: P<0.001), (**b**) un-accompanied by growth in inactive lever pressing. NVHL rats showed stronger acquisition (in terms of overall active lever pressing (***lesion: P<0.001) and shape of the acquisition curve (**lesion × days: P<0.01), while not differing from SHAMS on inactive lever pressing. Significance levels of Post-hoc One-Way ANOVAs on each day by lesion status are denoted directly above error bars (* P<0.05; **P<0.01; ***P<0.001). (**c**) NVHLs increased active lever time-out responding (**P<0.01) but not (**d**) time out-inactive lever pressing. Over acquisition, (**e**) NVHL rats achieved nicotine acquisition (20 days of ≥ 20 infusions/day) earlier than SHAMS (**P<0.01), accumulating (**f**) greater total nicotine intake (**P<0.01). Adolescent nicotine exposure (NIC groups) did not produce differential effects on these measures compared to adolescent saline exposure. Data depicted as means ± SEM.

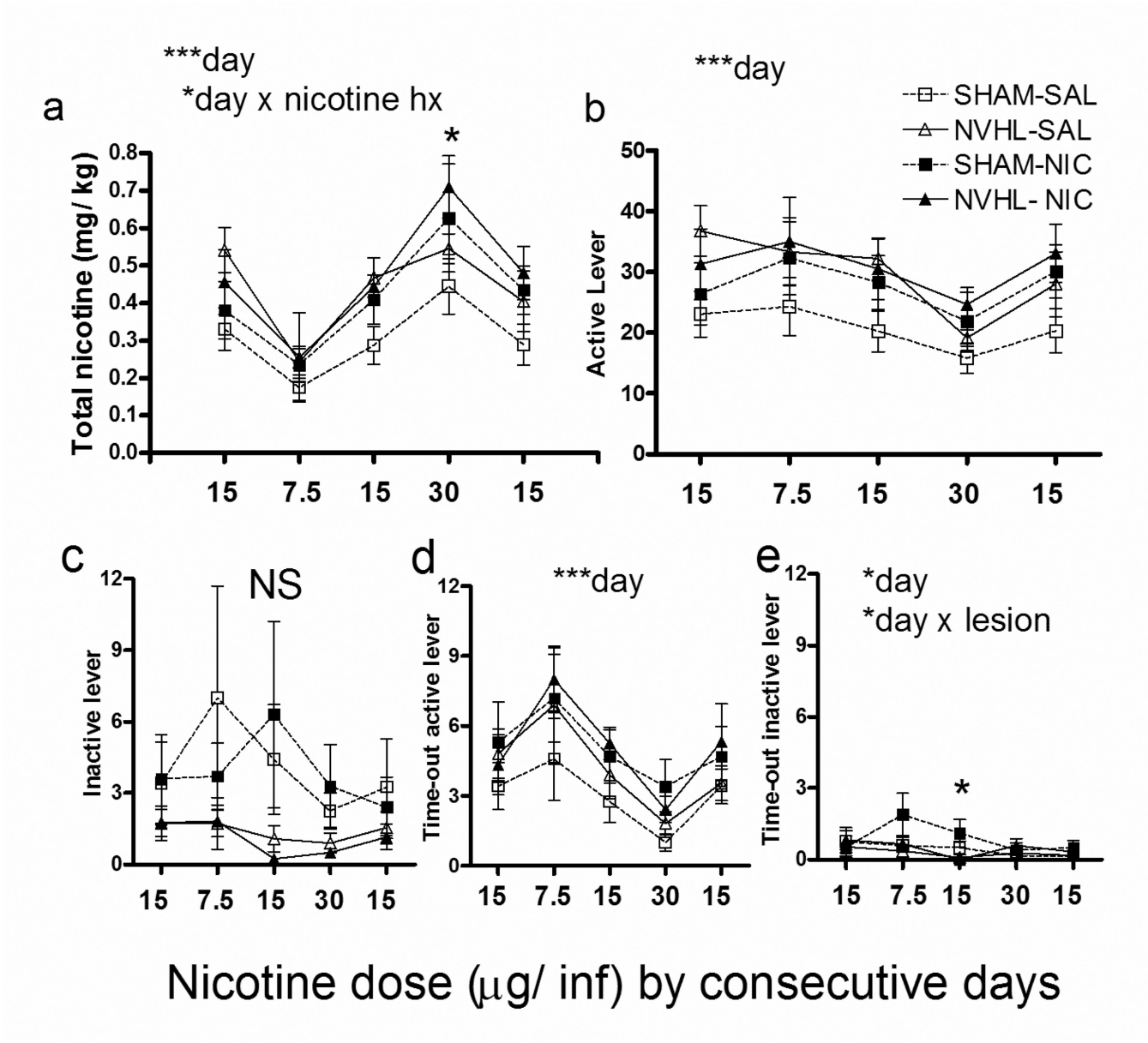

Figure 4.

Dose response testing after acquisition of nicotine self-administration, same subjects (n=45) as in Figs. 2 and 3. (**a**) Total nicotine intake varied significantly between days as the doses of nicotine infusions changed (***day: P<0.001), interacting with nicotine history in adolescence (*day × nicotine hx: P<0.05). This effect was carried by nicotine history-related increases in intake at the highest nicotine dose (One-way ANOVA by nicotine history: *P<0.05). **(b)** Active lever presses differed across days (***P<0.001). **(c)** Inactive lever pressing did not vary significantly whereas **(d)** Time-out active lever responding did vary by day (***P<0.001) as did **(e)** time-out inactive lever pressing (p<0.05) where sham rats showed greater responding in a dose dependent manner (*day × lesion:P<0.05) with shams

showing greater responding specifically at the middle 15 μg dose (post-hoc ANOVA by nicotine history: *P<0.05). Data are depicted as means ± SEM throughout all figures.

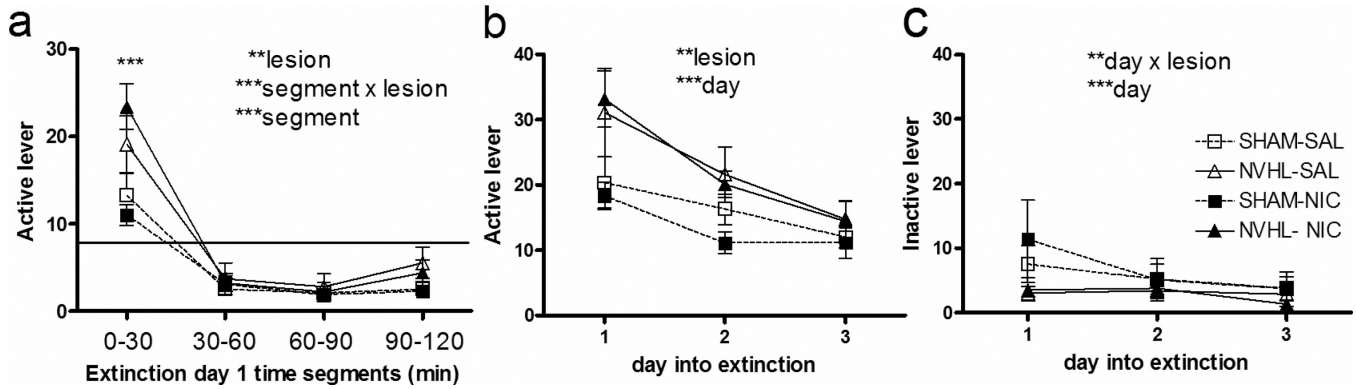

Figure 5.

Drug seeking measured by extinction phase lever pressing. For extinction day one, same subjects (n=45) are included as in Figs. 2,3 and 4 Subsequent extinction day analyses exclude rats that had met extinction criteria on previous days ((day 2: n=2 sham; n=1 NVHL; day 3: n=3 sham; n=2 NVHL) **(a)** During extinction day one, divided into 4 × 30 minute segments, an extinction burst marked by increased rates of active lever pressing in the first 30 minutes (compared to the 8.1 active lever presses per 30 minutes recorded in the prior nicotine reinforced session (horizontal line)) extinguished significantly over the next 90 minutes (***segment: P<0.001). Overall lesion effects (**lesion: P<0.01 and ***segment × lesion: P<0.001) to increase drug seeking were carried most prominently by increased pressing during the initial extinction burst (One-way by lesion status: P<0.001). (**b**) Over the first 3 extinction days on the previously nicotine paired lever, NVHLs again showed elevated drug-seeking (**P<0.01) superimposed on an extinguishing pattern (***day; P<0.001), while on the inactive lever (**c**), SHAM rats tended to have higher responding that habituated more significantly (**day × lesion: P<0.01; ***day: P<0.001). Unlike the dose response data, adolescent nicotine history effects had no effects on nicotine seeking during extinction.

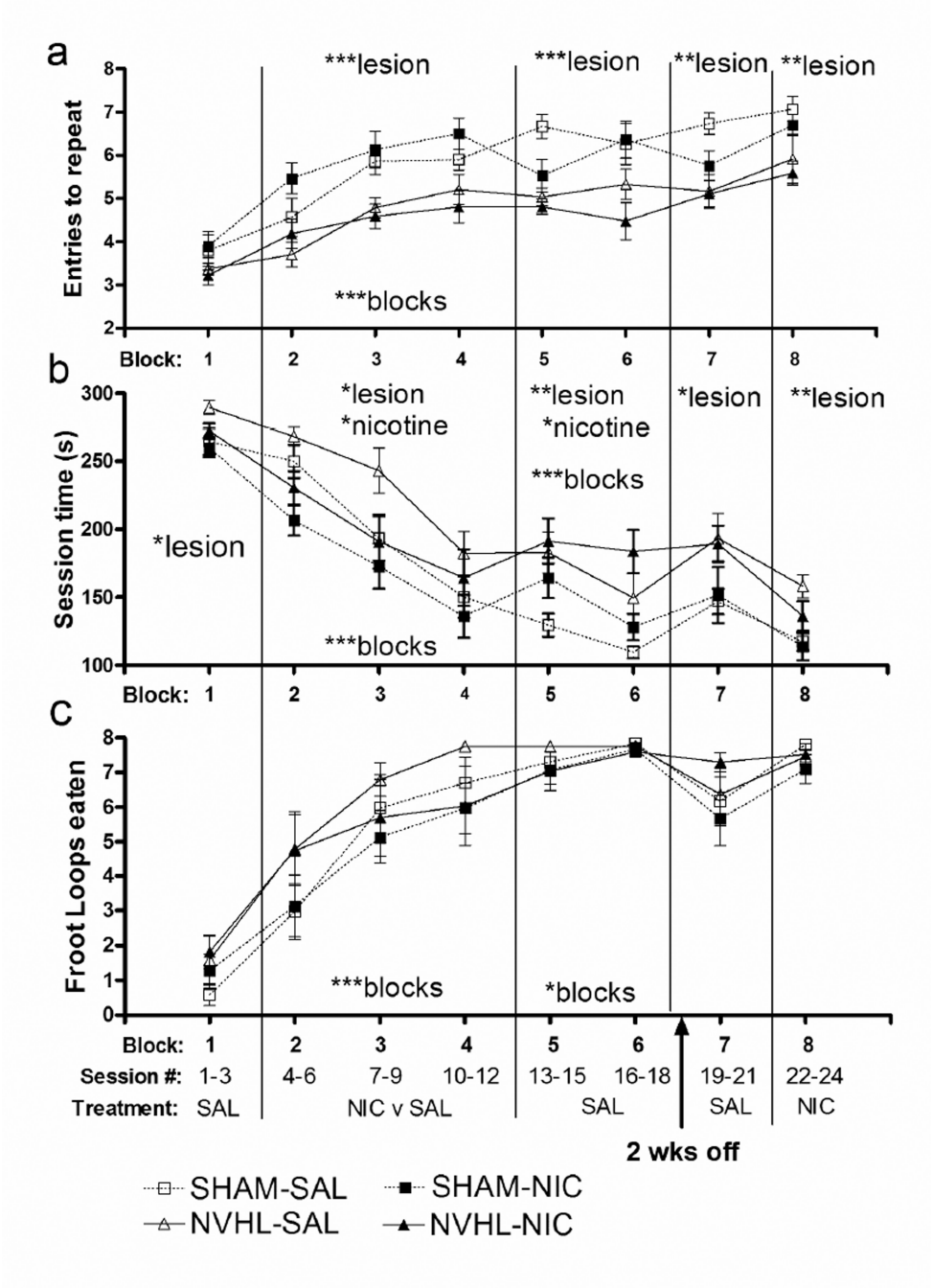

Figure 6.

RAM testing of spatial learning and working memory. Rats received 9 once daily nicotine (NVHL, n= 9; SHAM, n=10) vs. saline pre-injections (NVHL, n=8; SHAM, n=10) across blocks 2–4 with all receiving saline elsewhere except for the last block where they all received nicotine. In block 1, NVHL rats showed no impairments in (**a**) entries-to-repeat (ETR) but were (**b**) significantly slower in session time (*P<0.05). Across blocks 2–4, rats demonstrated learning with increased ETRs (***P<0.001), decreased session times (***P<0.001), and more **(c)** Froot Loops eaten (***P<0.001), but, NVHL rats showed

impaired cognition with lower ETRs (***P<0.001). Nicotine pre-injections produced no effects on ETR where the cognitive deficits due to NVHLs were most robust, but did improve cognitive efficiency in terms of reduced session time (*P<0.05). This improvement did not specifically reverse NVHL deficits on session time (*lesion: P<0.05). Over blocks 5–6, the recent nicotine exposure produced new cognitive deficits in terms of session time (*P<0.05) with NVHL deficits in ETR (*** P<0.001) and session time persisting (**P<0.01). In long-term recall ending with nicotine pre-injections (block 7- 8), NVHL deficits in ETR (block #7; **P<0.01; block #8:** P<0.01) and session time (block #7:* P<0.05; block #8: **P<0.01) also persisted, with no group differences in Fruit Loops consumed and no effects or interactions with prior nicotine pre-injections (blocks 2–4).

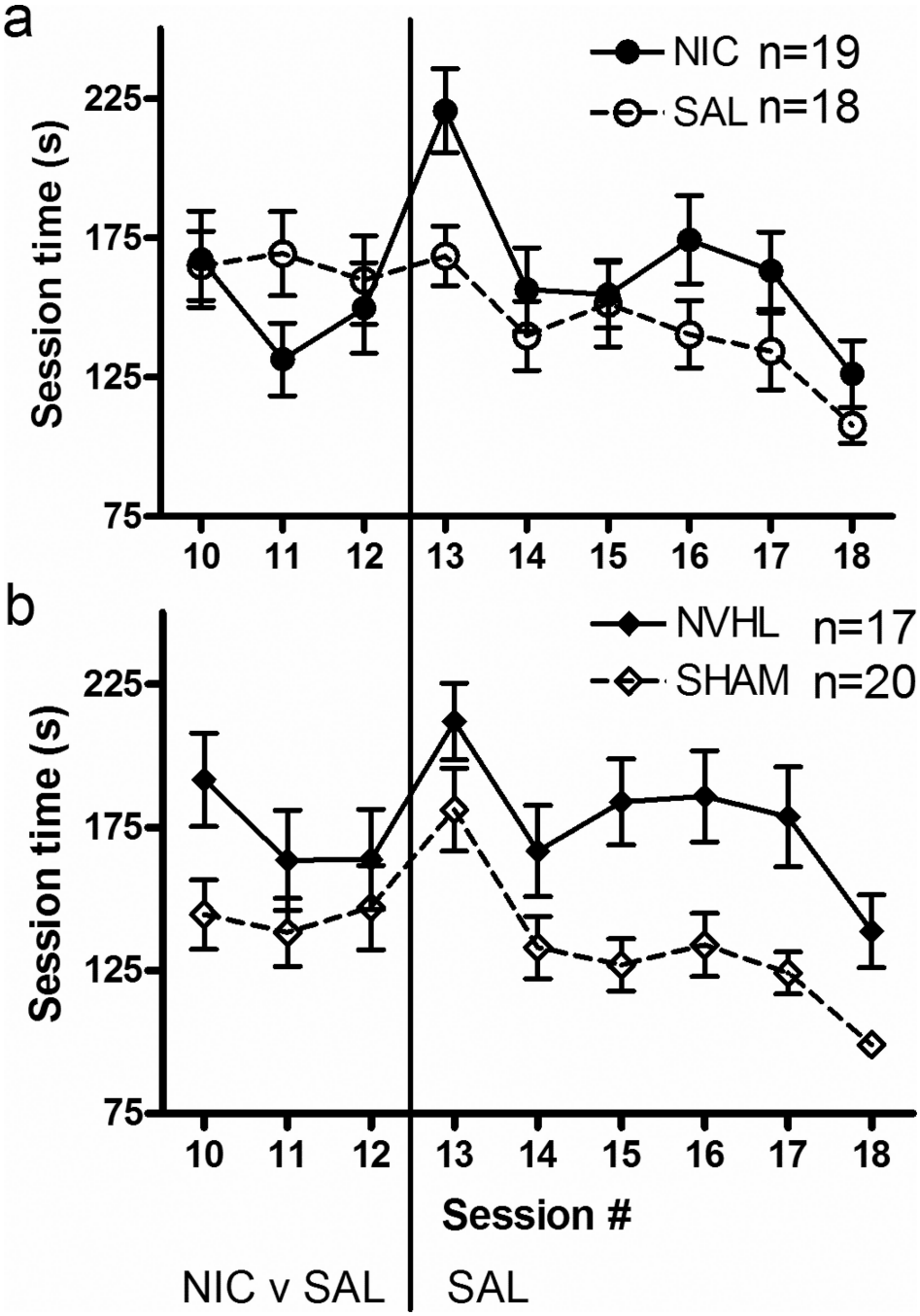

Figure 7.

RAM session times covering the transition from nicotine to saline pre-injections, same subjects (n=37) as in Fig. 6 with blocks 4, 5, and 6 decomposed into individual sessions for visual clarity. Comparison of rats according to **(a)** nicotine vs. saline exposure (during sessions 4–12) depict the detrimental cognitive effects of nicotine withdrawal. The comparison by **(b)** lesion status suggests how nicotine withdrawal impacts NVHL and Sham rats similarly.