Lesions of the ventral hippocampus attenuate the acquisition but not expression of sign-tracking behavior in rats

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Running Title: Ventral hippocampal lesions and sign-tracking behavior
Abstract: 251/300 words
Text Pages: 23
Figures: 6
Tables: 0

Keywords (5/5): Pavlovian conditioned approach; incentive salience; dopamine; norepinephrine; autoshaping

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Abstract

Individual variation in the attribution of motivational salience to reward-related cues is believed to underlie addiction vulnerability. Pavlovian conditioned approach measures individual variation in motivational salience by identifying rats that are attracted to and motivated by reward cues (sign-trackers) or motivationally fixed on the reward itself (goal-trackers). Previously, it has been demonstrated that sign-trackers are more vulnerable to addiction-like behavior. Moreover, sign-trackers release more dopamine in the nucleus accumbens than goal-trackers in response to reward-related cues, and sign- but not goal-tracking behavior is dopamine-dependent. In the present study, we investigated whether the ventral hippocampus, a potent driver of dopaminergic activity in the nucleus accumbens, modulates the acquisition and expression of Pavlovian conditioned approach behavior. In Experiment 1, lesions of the ventral, but not dorsal or total hippocampus, decreased sign-tracking behavior. In Experiment 2, lesions of the ventral hippocampus did not affect the expression of sign- or goal-tracking behaviors nor conditioned reinforcement. In addition, temporary inactivation of the ventral subiculum, the main output pathway of the ventral hippocampus, did not affect the expression of sign- or goal-tracking behaviors. High-pressure liquid chromatography of nucleus accumbens tissue punches revealed that ventral hippocampal lesions decreased levels of homovanillic acid and the homovanillic acid/dopamine ratio (a marker of dopamine release and metabolism) in only sign-trackers, and decreased accumbal norepinephrine levels in both sign- and goal-trackers. These results suggest that the ventral hippocampus is important for the acquisition but not expression of sign-tracking behavior, possibly as a result of altered dopamine and norepinephrine in the nucleus accumbens.

Introduction

It is important to understand why some individuals can try potentially addictive drugs without developing addiction, while others try the same drug and are quickly rendered incapable of controlling their urges to repeat the experience. Individual variation in the incentive-motivational salience of reward-related cues is believed to contribute to this individual

vulnerability to addiction (Flagel et al., 2009). Incentive-motivational salience is typically measured using a Pavlovian conditioned approach (PCA) procedure, during which a conditioned stimulus (CS; e.g., a lever) response-independently predicts the delivery of an unconditioned stimulus (US; e.g., a food pellet). During training, three behavioral phenotypes may emerge: sign-trackers (STs), which express primarily CS-directed conditioned responses (CRs), goal-trackers (GTs), which develop more US-directed CRs, and intermediate responders (IRs), which display both CRs. STs are more susceptible to cue-related, addiction-like behaviors than GTs or IRs, exhibiting more cue-induced reinstatement of drug-seeking (Saunders and Robinson, 2010; Yager et al., 2015) and even seeking drug cues despite adverse consequences (Saunders et al., 2013).

Understanding the brain regions that are activated during sign-tracking behavior is critical in determining the biological basis of individual variation in incentive-motivational salience and drug vulnerability. Previously, it has been demonstrated that food and drug cues activate an amygdalo-striatal-thalamic circuit in STs (Flagel et al., 2011a; Yager et al., 2015), and lesion studies have confirmed the importance of regions within this circuit (e.g., the nucleus accumbens [NAc], basolateral amygdala, and paraventricular nucleus of the thalamus) in the acquisition of sign-tracking behavior (Chang et al., 2012; Haight et al., 2015). Other regions with important connections to this circuit have been implicated in sign-tracking behavior as well, such as the hippocampus (HPC) (Ito et al., 2005), though little is known regarding how subregions of this heterogeneous structure individually regulate sign-tracking behavior.

The hippocampus can be broadly divided into dorsal and ventral regions, which have unique connectivity and functions (Fanselow and Dong, 2010). For example, efferents from the ventral HPC (vHPC), but not dorsal HPC (dHPC), are potent drivers of dopaminergic activity in the NAc, and lesions of the vHPC, but not dHPC, decrease dopamine (DA) levels in the NAc (Lipska et al., 1992; Lipska et al., 1991). Moreover, stimulation of the ventral subiculum (vSUB), the main output structure of the HPC, increases DA release in the NAc (Blaha et al., 1997; Taepavarapruk et al., 2000). Because STs release more DA in the NAc than GTs in response to reward-related cues, and sign-tracking, but not goal-tracking, behavior is DA-

dependent (Flagel et al., 2011b; Saunders and Robinson, 2012), it is possible that increased ventral hippocampal activity regulates NAc DA in STs, thereby enhancing the incentive-motivational value of reward-related cues. A reduction of activity in the vHPC would therefore be expected to decrease dopaminergic activity in response to reward-related cues and decrease sign-tracking behavior.

The present study aimed to determine how the HPC and its subregions contribute to the acquisition and expression of PCA behavior. We hypothesized that lesions of the vHPC, but not dHPC, would decrease sign-tracking behavior during the acquisition and expression of PCA behavior. In Experiment 1, rats received sham surgery or lesions of the vHPC, dHPC, or total HPC, before undergoing five daily sessions of PCA training. Based on our results from Experiment 1, in Experiment 2, rats underwent seven daily sessions of PCA training, followed by sham surgery or vHPC lesions, then tested for the expression of PCA behavior and conditioned reinforcement. Rats undergoing sham surgeries were implanted with cannulas targeting the vSUB, allowing us to temporarily inactivate and further probe the importance of the vHPC during the expression of PCA behavior. Following behavioral testing, tissue punches of the NAc were analyzed for monoamine and metabolite concentrations of DA as well as norepinephrine (NE) and serotonin (5-HT) using high-pressure liquid chromatography (HPLC).

Methods

Animals

Adult male Sprague Dawley rats (275-300g) were purchased from Harlan Laboratories and Charles River Laboratories to increase phenotypic diversity (Fitzpatrick et al., 2013). Rats were maintained on a 12:12-hr light/dark cycle, and food and water were available *ad libitum* for the duration of experimentation. Rats were acclimatized to the housing colony for two days prior to handling. All procedures were approved by the University Committee on the Use and Care of Animals (University of Michigan; Ann Arbor, MI).

Drugs

N-methyl-D-aspartate (NMDA; #M3262; Sigma-Aldrich, Inc.; St. Louis, MO), lidocaine hydrochloride (#L5647; Sigma-Aldrich, Inc.), and Pontamine Sky Blue (PSB; also known as Chicago Sky Blue 6B; #C8679); Sigma-Aldrich, Inc.) were used. NMDA was dissolved in sterile saline to make a 0.09 M solution (pH = 7.34-7.36). Lidocaine hydrochloride was dissolved in sterile saline to make a 20% solution (200 mg/mL; pH = 5.0), as previously described (Kantak et al., 2002). Sterile saline was used as the vehicle control.

Pavlovian Conditioned Approach: Apparatus

Modular conditioning chambers (24.1 cm width × 20.5 cm depth x 29.2 cm height; MED Associates, Inc.; St. Albans, VT) were used for Pavlovian conditioning. Each chamber was located in a sound-attenuating cabinet equipped with a ventilation fan to provide ambient white noise. Each chamber was equipped with a pellet magazine, an illuminated, retractable lever (counterbalanced on the left or right of the pellet magazine), and a red house light on the wall opposite of the pellet magazine. When inserted into the chamber, the retractable lever was illuminated by an LED light within the lever housing. A pellet dispenser delivered banana-flavored food pellets into the pellet magazine. An infrared sensor inside the pellet magazine measured head entries into the pellet magazine.

Pavlovian Conditioned Approach: Procedure

For two days prior to pretraining, rats were familiarized with banana-flavored food pellets (45mg; Bioserv; Frenchtown, NJ) in their home cages. On the third day, rats were placed into the chambers and underwent one pretraining session during which the red house light remained on but the lever was retracted. Fifty food pellets were delivered on a variable time (VT) 30-s schedule (i.e., one food pellet was delivered on average every 30 s, but actual delivery varied between 0-60 s). All rats consumed all the food pellets by the end of the pretraining session. Each trial during a PCA training session consisted of extension of the illuminated lever (the CS) into the chamber for 8 s on a VT 90-s schedule (i.e., one food pellet was delivered on average every 90 s, but actual delivery varied between 30-150 s). Retraction of the lever was

immediately followed by the response-independent delivery of one food pellet (the US) into the pellet magazine. Each test session consisted of 25 trials of CS-US pairings, resulting in a total session length of approximately 40 min. Each rat consumed all the food pellets that were delivered.

Conditioned Reinforcement: Procedure

For testing of conditioned reinforcement in Experiment 2, which lasted 40 min, each chamber was equipped with two nose-poke ports adjacent to a lever located in the center of the front wall of the operant chamber. Nose pokes into the active nose-poke port resulted in presentation of the lever-CS for 2 s on a fixed-ratio 1 (FR1) schedule, whereas nose pokes in inactive nose-poke port did not result in presentation of the lever-CS.

Surgery

For all surgical procedures, rats were administered carprofen (2 mg/kg; s.c.) for analgesia, anesthetized using a ketamine (90 mg/kg; i.p.) and xylazine (10 mg/kg; i.p.) solution, and placed in a stereotaxic frame (David Kopf Instruments; Tujunga, CA). For excitotoxic lesions, injectors (33-gauge; Plastics One, Inc.; Roanoke, VA), connected via PE-20 tubing to microsyringes (1 µL; Hamilton Company; Reno, NV) in a pump controller (Harvard Instruments; Holliston, MA) were lowered into the HPC. NMDA (0.09 M) was infused bilaterally. For dHPC infusions, 0.4 µL was infused at a rate of 0.4 µL/min per infusion site, and infusion cannulas were left in place for an additional 4 min before removal. For vHPC infusions, 0.2 µL was infused at a rate of 0.2 µL/min per infusion site, and infusion cannulas were left in place for an additional 2 min before removal. In Experiment 1, lesions were targeted at the dHPC (two sites; anterior-posterior [AP]: -2.8 mm, medial-lateral [ML]: ±1.6 mm, dorsal-ventral [DV]: -3.0 mm; AP: -4.2 mm, ML: ±2.6 mm, DV: -3.0 mm), vHPC (four sites: AP: -4.8 mm, ML: ±4.8 mm, DV: -6.0 mm; AP: -5.3 mm, ML: ±4.6 mm, DV: -4.2 mm; AP: -5.3 mm, ML: ±4.6 mm, DV: -6.0 mm; AP: -5.8 mm, ML: ±4.6 mm, DV: -4.2 mm), or total HPC (a combination of the two previously described lesions for a total of six sites). AP coordinates were measured from bregma,

DV coordinates were measured from dura, and all coordinates were referenced to Paxinos and Watson (2007). In Experiment 1, surgical controls received incisions and burr holes on the skull over the HPC, but no infusions were performed. In Experiment 2, surgical controls were implanted bilaterally with guide cannulas (26-gauge, cut 7 mm below the pedestal; Plastics One, Inc.) targeted at the vSUB (AP: -5.6 mm, ML: ±4.6 mm, DV: -5.8 mm). Injection cannulas that would later be inserted into these guide cannulas were 8 mm long, overhanging 1 mm past the guide cannula tip. Two jeweler's screws were secured both anterior and posterior to the burr holes, and dental acrylic (Fastray™; Harry J. Bosworth Company; Skokie, IL) was applied around the guide cannulas and screws to form a head stage. Dummy cannulas (8 mm, 1 mm overhang; Plastics One, Inc.) were inserted into the guide cannulas and removed only during testing. Following all surgeries, rats recovered for seven days prior to behavioral testing with food and water available *ad libitum*.

Experiment 1: Procedure

Rats underwent pre-conditioning hippocampal lesions (sham, dHPC, vHPC, or total HPC) and, following the surgical recovery period, five daily PCA training session to determine the effect of hippocampal lesions on the acquisition of PCA behavior. Following the last behavioral session, rats were transcardially perfused with a 4% paraformaldehyde solution (PFA; in 0.1 M phosphate-buffered saline; pH = 7.34-7.36). Brains were removed, immediately post-fixed in 4% PFA for one hour, and transferred to a 20% sucrose solution until saturated. Next, tissues were sectioned on a cryostat (35 µM; Leica CM1850; Leica Microsystems, Inc.; Buffalo Grove, IL), stained with Cresyl Violet (#C5042; Sigma-Aldrich, Inc.), and used for histological verification of lesions. Unilateral lesions were excluded from analysis (vHPC, n = 2; dHPC, n = 4, total HPC, n = 2).

Experiment 2: Procedure

Rats underwent seven daily PCA training sessions before hippocampal lesions (sham and vHPC). In this experiment, surgical controls were implanted with bilateral cannulas targeted at

the vSUB. Following the seven-day surgical recovery period, rats underwent an additional seven daily PCA training sessions to determine the effect of post-conditioning lesions on the expression of PCA behavior. Twenty-four hours later, rats underwent two more daily PCA training sessions during which surgical controls received bilateral infusions (0.5 μL/side) of a 20% lidocaine solution (100 μg/side; in sterile saline) or vehicle at an infusion rate of 0.5 μL/min for 1 min in a counter-balanced manner (i.e., rats received lidocaine or saline during each session in a counter-balanced manner). For statistical analysis and graphical presentation, both sessions were collapsed into vehicle and lidocaine groups. Infusion cannulas were left in place for an additional minute to allow diffusion, and rats immediately underwent testing. Lesion rats also underwent two PCA training sessions to ensure that all subjects received equal amounts of PCA training. Twenty-four hours later, all rats underwent a test for conditioned reinforcement. Five days after the conditioned reinforcement test, sham rats received bilateral infusions (0.5 μL/side) of a 2% PSB solution at a flow rate of 0.5 μL/min to dye and visualize infusion sites in the vSUB. Infusion cannulas were left in place for an additional minute to allow diffusion. Next, all rats were rapidly decapitated and brains were removed and placed into an ice-cold adult rat brain matrix. A 1-mm thick tissue section including the NAc (AP: +1.2 mm) was biopsied using 2-mm diameter tissue biopsy punches. Whole brains posterior to this section were kept for histological verification of lesions and cannula placements. All tissue was immediately flash-frozen in isopentane (Sigma Aldrich, Inc.) over dry ice. Tissue punches were later processed to quantify levels of the following monoamines and their metabolites: DA, 3,4-dihydrophenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytryamine (3-MT), NE, 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA). Tissue sections were stained with Cresyl Violet (Sigma-Aldrich, Inc.) and used for histological verification. For lesions, unilateral lesions were excluded from further analysis (ST, n = 2). For cannula placement, subjects with an absence of cannula tracks and dye were kept for statistical comparison between lesion and sham control; however, they were excluded from further analysis for testing of temporary inactivation of the vSUB (GT, n = 1; ST, n = 2).

High-pressure liquid chromatography (HPLC)

Frozen tissue samples were weighed and sonically disrupted in 100 µL 0.2N $HCLO_4$. Samples were then centrifuged at 4°C at 12,100 g for 10 min. A 50 µL aliquot of supernatant was removed and monoamine analysis was performed utilizing a Dionex Ultimate 3000 UHPLS system (Thermo Fisher Scientific, Inc.; Waltham, MA). At a flow rate of 0.6 mL/min, 10 µL of each sample was injected with an auto-sampler maintained at 4°C and with a 100 µL sample loop onto a C18-RP (2-µL diameter) column maintained at 25°C. Test Mobile Phase (Thermo Fisher Scientific, Inc.) containing acetonitrile, phosphate buffer, and an ion-pairing reagent was used. Coulometric detection was achieved with an ultra-analytical dual electrode cell (Thermo Fisher Scientific, Inc.) set at -175 mV (reference electrode) and 300 mV (working electrode). Gain settings for both electrodes were set to 100 µA. A guard cell set to 350 mV and guard column (2.1/3.0 mm ID; Thermo Fisher Scientific, Inc.) were also used. Monoamine and metabolite levels were quantified by comparison to external standards (Sigma-Aldrich, Inc.) ran in sequence with tissue samples and corrected for tissue weight. The order of sample analysis within the run was randomized. Chromatograms were obtained and analyzed using Chromeleon 7 Chromatography Data System software (Dionex Corp.; Sunnyvale, CA) with peak heights being the output measure. The detection threshold was set at 5 nA, and samples with peak heights lower than 5 nA were excluded from analysis. Monoamine and metabolite levels (DA, DOPAC, HVA, 3-MT, NE, 5-HT and 5-HIAA) were expressed as absolute tissue values (ng neurochemical / mg wet tissue weight; ng/mg).

Statistical Analysis

PCA behavior was scored using an index that incorporates the number, latency, and probability of lever presses (sign-tracking CR) and magazine entries (goal-tracking CR) during CS presentations within a session (Meyer et al., 2012). Briefly, we averaged together the response bias (i.e., [number of lever presses – number of magazine entries] / [lever presses + magazine entries]), latency bias (i.e., [magazine entry latency – lever press latency]/8), and probability difference (i.e., lever press probability – magazine entry probability). The PCA index

scores behavior from +1.0 (absolute sign-tracking) to -1.0 (absolute goal-tracking), with 0 representing no bias. When applicable, the average PCA index scores of Sessions 6 and 7 were used to classify rats as STs (score $\geq$ 0.5), GTs (score $\leq$ -0.5), and IRs (-0.5 < score < 0.5). In Experiment 2, IRs were excluded from further experimentation and statistical analysis. For conditioned reinforcement, active – inactive nose-poke port responses were quantified and compared between groups.

SPSS (Version 22; IBM, Inc.) was used for all statistical analyses. For all linear mixed models, the covariance structure was selected based upon Akaike's information criterion (i.e., the lowest number criterion using a given covariance structure represents the highest quality statistical model; Akaike, 1974). In Experiment 1, PCA behavior across training sessions was analyzed using a linear mixed model with an autoregressive (AR1) covariance structure with Lesion (sham, vHPC, dHPC, total HPC) as the main factor. In Experiment 2, PCA behavior across training sessions was analyzed using a linear mixed model (AR1 covariance structure) with Phenotype (GT and ST) and Lesion (sham and vHPC) as factors. For the test of temporary inactivation, PCA behavior was analyzed using a three-way analysis of variance (ANOVA) with Phenotype (GT and ST), Drug (Saline and Lidocaine), and Order (First and Second) as factors. For the conditioned reinforcement test, nose-poke port responses (active – inactive) were analyzed using a two-way ANOVA with Phenotype (GT and ST) and Lesion (sham and vHPC) as factors. For HPLC analysis, neurochemicals were analyzed using a two-way ANOVA with Lesion (sham and vHPC) and Phenotype (GT and ST) as factors. With a significant ANOVA, multiple comparisons were performed using Fisher's Least Significant Difference (LSD) post hoc test.

Results

Experiment 1

Excitotoxic lesions of the hippocampus (sham, n = 13; vHPC, n = 11; dHPC, n = 8; total HPC, n = 10) were performed prior to five daily PCA training sessions to determine whether the lesions affected the acquisition of PCA behavior. Figure 1 shows a schematic representation of

the extent of NMDA-induced lesions in the vHPC, dHPC, and total HPC. Figure 2 shows that there was an effect of Lesion on lever press number ($F_{(3,38)} = 2.85$, $p = 0.05$), latency ($F_{(3,38)} = 3.34$, $p = 0.029$), and probability ($F_{(3,38)} = 3.31$, $p = 0.03$) as well as magazine entry number ($F_{(3,38)} = 3.01$, $p = 0.042$), and latency ($F_{(3,38)} = 3.55$, $p = 0.023$), which were averaged over Sessions 4 and 5. There was no effect of Lesion on magazine entry probability ($F_{(3,38)} = 2.76$, $p = 0.055$). Post hoc comparisons revealed that, compared to the sham condition, vHPC lesions decreased lever press number ($p = 0.007$), latency ($p = 0.009$), and probability ($p = 0.006$) as well as increased magazine entry number ($p = 0.019$) and latency ($p = 0.007$). Lesions of the dHPC, compared to the sham condition, only increased magazine entry number ($p = 0.02$) and latency ($p = 0.017$). Lesions of the total HPC, compared to the sham condition, had no effect on any variable.

There was an interaction of Lesion and Session on lever press number ($F_{(16,148.84)} = 2.95$, $p = 3.06 \times 10^{-4}$), latency ($F_{(16,143.48)} = 2.99$, $p = 2.59 \times 10^{-4}$), and probability ($F_{(16,142.65)} = 2.70$, $p = 0.001$) as well as magazine entry number ($F_{(16,139.94)} = 3.89$, $p = 5.0 \times 10^{-6}$), latency ($F_{(16,138.61)} = 4.26$, $p = 1.0 \times 10^{-6}$), and probability ($F_{(16,138.09)} = 4.11$, $p = 2.0 \times 10^{-6}$) (Figure 3). Post-hoc comparisons revealed that, compared to the sham condition, vHPC lesions decreased lever press number ($ps < 0.01$), latency ($ps < 0.01$), and probability ($ps < 0.01$) during Sessions 3-5 as well as increased magazine entry number ($ps < 0.05$), latency $ps < 0.05$), and probability ($ps < 0.05$) during Sessions 4-5. dHPC lesions, compared to the sham condition, increased magazine entry number ($ps < 0.05$), latency ($ps < 0.05$), and probability ($ps < 0.05$) during Sessions 3-5. Lesions of the total HPC, compared to the sham condition, had no effect on any variable. Figure 3 shows that there was an interaction of Lesion and Session on PCA index scores ($F_{(16,142.85)} = 3.02$, $p = 2.33 \times 10^{-4}$). Post-hoc comparisons revealed that only vHPC lesions, compared to the sham condition, decreased PCA index score ($ps < 0.01$) during Sessions 3-5. Lastly, there was no effect of Lesion on non-CS magazine entries over Sessions 1-5 (data not shown; $F_{(3,61.75)} = 0.80$, $p = 0.50$).

Experiment 2

Before surgery, rats underwent PCA training and were classified as STs, GT, and IRs; however, only STs (n = 24) and GTs (n = 15) were used for further experimental testing. During seven daily PCA training sessions, STs and GTs differed in their lever press number ($F_{(1,37.63)}$ = 29.01, p = 4.0 x 10^{-6}), latency ($F_{(1,37.17)}$ = 37.71, p = 3.98 x 10^{-7}), and probability ($F_{(1,36.03)}$ = 48.61, p = 3.56 x 10^{-8}) as well as their magazine entry number ($F_{(1,47.15)}$ = 43.17, p = 3.62 x 10^{-8}), latency ($F_{(1,46.28)}$ = 54.81, p = 2.19 x 10^{-9}), and probability ($F_{(1,44.76)}$ = 53.70, p = 3.44 x 10^{-9}). STs and GTs differed on their PCA index scores over the seven daily PCA training sessions (Figure 5*A*; $F_{(1,40.10)}$ = 74.51, p = 1.10 x 10^{-10}), and the average PCA index scores of Sessions 6 and 7 were used to determine PCA phenotypes. Following training, rats underwent vHPC lesions (Figure 4*A*; ST, n = 12; GT, n = 6) or sham surgeries (ST, n = 12; GT, n = 9), which included cannulas targeted at the vSUB (Figure 4*B*). Following a surgical recovery period of seven days, rats underwent an additional seven daily PCA training sessions to determine the effect of vHPC lesions on the expression of PCA behavior. In GTs, there was no effect of Lesion on lever press number ($F_{(1,12.89)}$ = 0.51, p = 0.49), latency ($F_{(1,13.95)}$ = 2.58, p = 0.40), and probability ($F_{(1,13.86)}$ = 0.63, p = 0.44), or magazine entry number ($F_{(1,14.24)}$ = 1.55, p = 0.23), latency ($F_{(1,17.49)}$ = 0.58, p = 0.46), and probability ($F_{(1,15.86)}$ = 0.50, p = 0.49). Similarly, in STs, there was no effect of Lesion on lever press number ($F_{(1,23.19)}$ = 0.51, p = 0.48), latency ($F_{(1,22.37)}$ = 0.02, p = 0.88), and probability ($F_{(1,20.1)}$ = 0.71, p = 0.79) or magazine entry number ($F_{(1,19.51)}$ = 0.001, p = 0.97), latency ($F_{(1,24.28)}$ = 0, p = 1.0), and probability ($F_{(1,20.9)}$ = 0.024, p = 0.88). Furthermore, Figure 5*B* shows that there was no effect of Lesion on PCA index scores in GTs ($F_{(1,14.29)}$ = 0.88, p = 0.36) or STs ($F_{(1,17.62)}$ = 4.0 x 10^{-5}, p = 1.0).

Twenty-four hours after the final session of PCA training, sham controls were infused bilaterally in the vSUB with a 20% lidocaine solution or vehicle and tested in a counter-balanced manner across two PCA training sessions, which served as the test of temporary inactivation. Figure 4*B* shows a schematic representation of injection sites in the vSUB. Figure 5*C* shows that temporary inactivation of the vSUB did not affect the expression of PCA behavior in GTs (effect of Drug; $F_{(1,9)}$ = 0.02, p = 0.89) or STs (effect of Drug; $F_{(1,13)}$ = 0.91, p = 0.36). In addition, there was no effect of Order (i.e., receiving lidocaine infusion on the first test session and vehicle

infusion on the second test session or vice versa; $F_{(1,22)} = 0.007$, $p = 0.93$). Rats with vHPC lesions underwent two PCA training sessions as well to ensure that all rats received similar behavioral testing procedures. Twenty-four hours later, all rats underwent a test for conditioned reinforcement to determine whether vHPC lesions affected the conditioned reinforcing properties of the lever. As expected, Figure 5*D* shows that STs exhibit greater conditioned reinforcement than GTs (i.e., higher active – inactive nose-poke port responses; effect of Phenotype; $F_{(1, 32)} = 6.28$, $p = 0.018$). However, vHPC lesions did not affect conditioned reinforcement in GTs (effect of Lesion; $F_{(1,13)} = 0.91$, $p = 0.36$) or STs (effect of Lesion; $F_{(1, 14)} = 0.51$, $p = 0.49$).

Using tissue punches of the NAc taken five days after the completion of testing (Figure 4*C*), HPLC analysis revealed an effect of Lesion on HVA levels (Figure 6*A*; $F_{(1,30)} = 7.63$, $p = 0.0097$) and HVA/DA (a marker of DA release and metabolism; Figure 6*B*; $F_{(1,30)} = 6.90$, $p = 0.013$). Post hoc comparisons showed that lesions decreased HVA levels in STs ($p = 0.0014$), but not GTs ($p = 0.51$), and decreased HVA/DA in STs ($p = 0.011$), but not GTs ($p = 0.26$). In addition, there was an effect of Lesion on NE levels across phenotypes (Figure 6*C*; $F_{(1,30)} = 4.21$, $p = 0.049$). There was no effect of Lesion (data not shown) on levels of DA ($F_{(1,30)} = 0.008$, $p = 0.93$), DOPAC ($F_{(1,30)} = 0.004$, $p = 0.95$), 3-MT ($F_{(1,30)} = 0.97$, $p = 0.33$), 5-HT ($F_{(1,30)} = 1.37$, $p = 0.25$), or 5-HIAA ($F_{(1,30)} = 0.22$, $p = 0.64$).

Discussion

The present study investigated the role of the HPC in the acquisition and expression of PCA behavior. In Experiment 1, we found that vHPC lesions decreased sign-tracking behavior and increased goal-tracking behavior during the acquisition of PCA training. Moreover, dHPC lesions also increased goal-tracking behavior, while total HPC lesions had no effect. Consequently, only the vHPC was further investigated in Experiment 2. During this experiment, we demonstrated that neither vHPC lesions nor temporary inactivation of the vSUB, the main output structure of the vHPC, affected the expression of PCA behavior. Post-conditioning lesions of the vHPC also did not affect conditioned reinforcement. Finally, lesions of the vHPC

decreased NE across both phenotypes, and decreased HVA and HVA/DA (a marker of DA release and metabolism) in STs, but not GTs.

In agreement with our hypothesis, vHPC lesions decreased the acquisition of sign-tracking (and also increased the acquisition of goal-tracking) behavior in rats. vHPC lesions may have decreased sign-tracking behavior by attenuating activity of dopaminergic cell bodies in the VTA (Floresco et al., 2001) and/or VTA terminals in the NAc or their terminals in the NAc (Blaha et al., 1997). Alternatively, the loss of glutamatergic input from the vHPC to medium spiny neurons in the NAc may have also decreased sign-tracking behavior by disrupting gating and outflow of NAc signals (French and Totterdell, 2002).

Interestingly, total HPC lesions did not have a similar effect as vHPC lesions, even though the region is included in the total HPC lesion. Previously, it has been reported that neonatal vHPC-induced amphetamine hyperlocomotion is abolished if the lesion encompasses both the vHPC and dHPC, similar to our total HPC lesion (Swerdlow et al., 2001). The authors suggested that the larger lesions may diminish a complex inhibitory control from the vHPC to dHPC that is critical for vHPC-induced behavioral alterations. A similar phenomenon may have occurred with our total HPC lesions, although future experiments, such as disconnection procedures, would need to be performed to investigate this question.

Unexpectedly, dHPC lesions also increased the acquisition of goal-tracking behavior. The dHPC has a role in the predictive value of a CS during Pavlovian conditioning (Munera et al., 2001) and cue-reward associations (Jacquet et al., 2013); therefore, it may seem counterintuitive that dHPC lesions increased goal-tracking behavior. It is possible, however, that neural activity in the dHPC competes with other brain regions during the acquisition of goal-tracking behavior, and dHPC lesions release these regions, facilitating goal-tracking behavior. For example, in an appetitive conditioned cue preference procedure, it has been suggested that competition between the HPC and lateral amygdala balances learning strategies with the lateral amygdala facilitating approach behavior to the site of food delivery in the absence of hippocampal activity (Chai and White, 2004; Gaskin and White, 2006).

In addition, our data seem to be at odds with previous reports that total HPC lesions increase sign-tracking behavior (Ito et al., 2005). It is possible that this incongruence arises from experimental differences in CS-US proximity (Christie, 1996), CS modality (Beckmann and Chow, 2015), or even rat strain differences in lesion-induced behavioral alterations (Lipska and Weinberger, 1995; Wood et al., 2001). However, the most likely explanation is that the PCA procedure employed by Ito et al. involves discrimination between a CS+ and CS- that are identical except for their physical location on the left or right of the wall. Rats in their study were required to be at the opposite side of the testing chamber before the start of each trial, which ensured that the CS+ was always on the same side of their body. Therefore, to discriminate between the two stimuli, the rats could either identify the actual location of the CS within the chamber, in a hippocampal-dependent manner, or simply identify whether the CS was on the left or right of their visual field. The latter strategy is both more efficient and hippocampal-independent, so elimination of competition from slower hippocampal-dependent processes can actually facilitate performance of such a task (Sanderson et al., 2012). The PCA procedure in the present study did not involve discrimination and would therefore not be expected to improve in this manner with hippocampal damage.

Following PCA training, vHPC lesions did not affect the expression of sign- or goal-tracking behaviors. In addition, vHPC lesions did not affect the conditioned reinforcing properties of the lever, although, consistent with previous findings, STs displayed higher conditioned reinforcement than GTs (Robinson and Flagel, 2009). Similarly, temporary inactivation of the vSUB did not affect the expression of either sign- or goal-tracking behaviors. Previously, it has been demonstrated that sign-tracking behavior becomes DA-independent after sufficient training (Clark et al., 2013; Darvas et al., 2014). Therefore, if the vHPC facilitates DA release in the NAc during PCA behavior, it may only be required during acquisition, and not expression following extended training, during which dopaminergic activity is no longer required for the maintenance of conditioned responding.

Our neurochemical results are in agreement with previous findings that HPC lesions decrease DA metabolites and NE in the NAc (Springer and Isaacson, 1982). Interestingly, we

demonstrated that vHPC lesions attenuate HVA and HVA/DA in STs but not GTs. Individual differences in the functional connectivity between the HPC, NAc, and ventral tegmental area (VTA) have been demonstrated in humans (Kahn and Shohamy, 2013), and differential connectivity within these regions may have resulted in our observed differences in HVA and HVA/DA following vHPC lesions. In the current experiment, we were unable to determine through what pathways the vHPC influences dopaminergic activity in the NAc; however, it is known that the vHPC modulates both dopaminergic cell bodies of the VTA, which project to the NAc (Floresco et al., 2001), and VTA terminals in the NAc (Blaha et al., 1997). With regard to NE, the NAc receives primary noradrenergic input from the A1 cell group of the nucleus tractus solitarius (NTS) in the ventral medulla (Delfs et al., 1998). Although the vHPC does not directly innervate the NTS, it can regulate NTS activity through an indirect circuit involving the infralimbic cortex, which projects directly to the NTS (Fisk and Wyss, 2000; Ruit and Neafsey, 1990). Transection of the ventral noradrenergic bundle, which contains noradrenergic projections from the NTS, decreases DA turnover in the NAc (O'Donohue et al., 1979). Therefore, the decreases in NE levels and DA turnover may be interconnected, especially given the fact that individual differences have been identified in the ability of noradrenergic compounds to modulate DA in the NAc (Tuinstra and Cools, 2000). Alternatively, it is possible that tissue punches included adjacent NE-rich regions, such as the ventral pallidum and bed nucleus of the stria terminalis, which are contiguous with the NAc (Berridge et al., 1997), receive noradrenergic innervation from the locus coeruleus (Jones and Yang, 1985), and could have contributed to observed differences in NE levels.

In summary, preconditioning lesions of the vHPC, but not dHPC or total HPC, decreased sign-tracking behavior while increasing goal-tracking behavior during the acquisition of PCA behavior. Moreover, post-conditioning lesions of the vHPC did not affect the expression of PCA behavior or conditioned reinforcement. In addition, vHPC lesion-induced alterations in DA turnover and NE levels in the NAc may underlie the observed changes in PCA behavior. These results demonstrate that the vHPC is critical for the acquisition, but not expression, of sign-

tracking behavior and adds to a growing body of literature indicating that the vHPC modulates motivated behavior and vulnerability to addiction (Jasinska et al., 2014; Robbins et al., 2008).

Acknowledgements

This work was funded by the University of Michigan Department of Psychiatry (U032826; JDM), Wayne State University Department of Psychiatry and Behavioral Neurosciences (SAP), and Department of Defense National Defense Science and Engineering Graduate (NDSEG) Fellowship (CJF).

Figures and Legends

Figure 1. Schematic representation of NMDA lesions of the (A) dorsal, (B) ventral, and (C) total hippocampus. Cresyl violet-stained sections were visualized underneath a light microscope, and the largest (dark gray) and smallest (light gray) lesions were identified. Coronal sections are presented from 1.88 to 6.30 mm posterior to bregma (adapted from Paxinos and Watson, 2007).

Figure 2. Before Pavlovian conditioned approach training, surgeries were performed to lesion the ventral, dorsal, or total hippocampus. After surgical recovery, rats underwent five daily sessions of PCA training, and the number, latency, and probability of lever presses and magazine entries were averaged over Sessions 4 and 5. Data are presented as mean + S.E.M. * - $p < 0.05$, ** - $p < 0.01$.

Figure 3. Pavlovian conditioned approach (PCA) index scores of rats that underwent ventral, dorsal, or total hippocampal lesions before the acquisition of PCA training. Data are presented as mean ± S.E.M. ** < 0.01, compared to the sham group.

Figure 4. Schematic representation (adapted from Paxinos and Watson, 2007) of (A) NMDA-induced lesions of the ventral hippocampus (coronal sections presented from 4.52 to 6.30 mm posterior to bregma), (B) cannulas bilaterally targeted at the ventral subiculum (coronal sections

presented from 5.30 to 6.30 mm posterior to bregma), and (C) tissue punches taken from the nucleus accumbens (coronal section presented at 1.20 anterior to bregma). For ventral hippocampal lesions, the largest (dark gray) and smallest (light gray) lesions were identified. For cannula placements, black circles represent the location of the infusion cannula tip. For tissue punches, the location of the 2-mm tissue punch is shaded (light gray).

Figure 5. (A) Rats underwent seven daily sessions of Pavlovian conditioned approach (PCA) training, and the average PCA index scores of Sessions 6 and 7 were used to phenotype STs and GTs. (B) After performing surgeries to lesion the ventral hippocampus (Lesion) or implant guide cannulas targeted at the ventral subiculum (Sham), rats underwent another seven daily sessions of PCA training. (C) Next, sham rats were infused with either lidocaine (20% solution) or saline in a counter-balanced manner immediately before two additional PCA training sessions, which served as a temporary inactivation test. (D) Finally, all rats underwent a conditioned reinforcement test during which active but not inactive nose-poke (NP) responses resulted in 2-s presentations of the lever. Data are presented as mean ± S.E.M. * - $p < 0.05$.

Figure 6. Five days following the last test session (conditioned reinforcement), tissue punches of the nucleus accumbens were taken to measure levels of monoamines and their metabolites using HPLC. Significant differences between lesion (ventral hippocampus) and sham rats were observed with (A) homovanillic acid (HVA), (B) the ratio of HVA to dopamine (DA), and (C) norepinephrine (NE). Data are presented as mean + S.E.M. * - $p < 0.05$, ** - $p < 0.01$.

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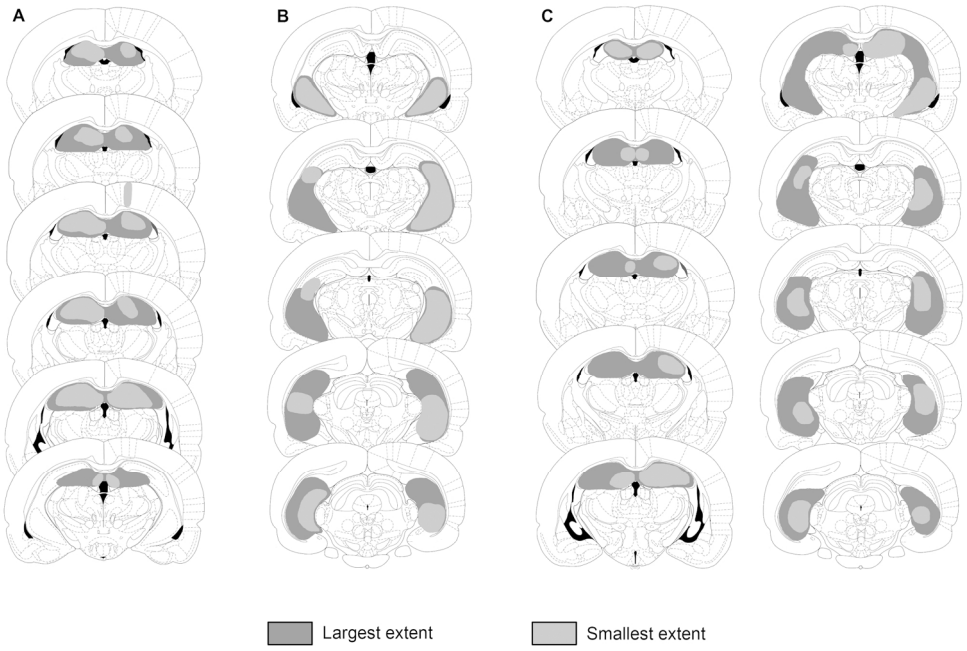

Figure 1. Schematic representation of NMDA lesions of the (A) dorsal, (B) ventral, and (C) total hippocampus. Cresyl violet-stained sections were visualized underneath a light microscope, and the largest (dark gray) and smallest (light gray) lesions were identified. Coronal sections are presented from 1.88 to 6.30 mm posterior to bregma (adapted from Paxinos and Watson, 2007).
Figure 1
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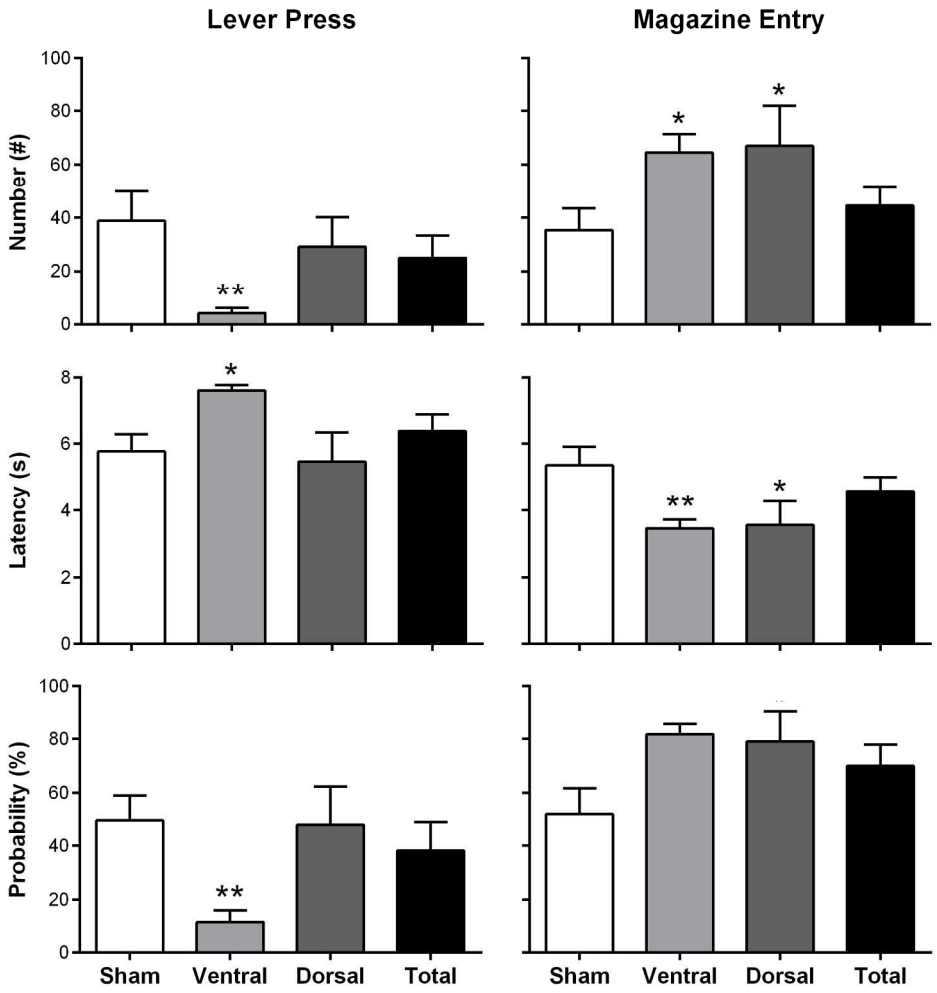

Figure 2. Before Pavlovian conditioned approach training, surgeries were performed to lesion the ventral, dorsal, or total hippocampus. After surgical recovery, rats underwent five daily sessions of PCA training, and the number, latency, and probability of lever presses and magazine entries were averaged over Sessions 4 and 5. Data are presented as mean + S.E.M. * - $p < 0.05$, ** - $p < 0.01$.
Figure 2
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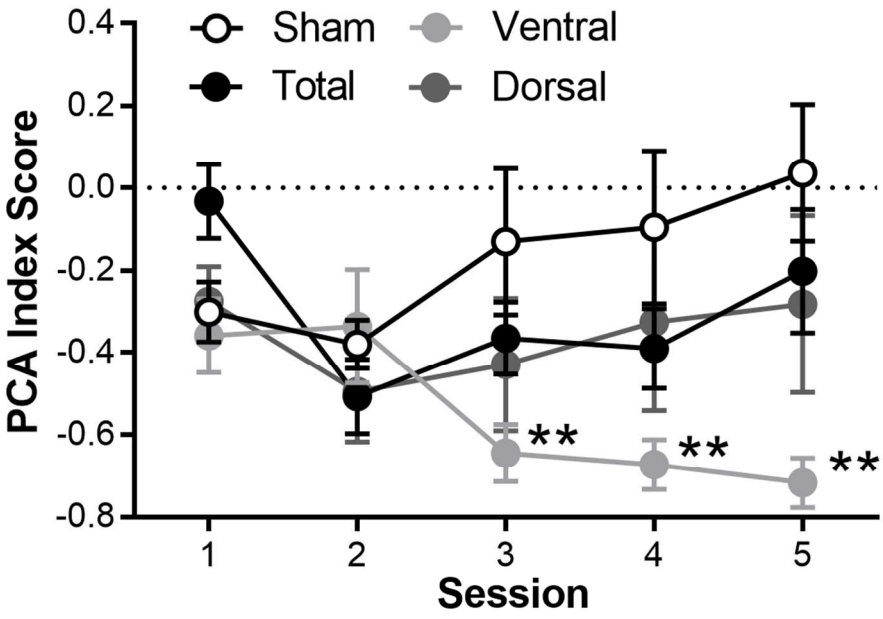

Figure 3. Pavlovian conditioned approach (PCA) index scores of rats that underwent ventral, dorsal, or total hippocampal lesions before the acquisition of PCA training. Data are presented as mean ± S.E.M. ** < 0.01, compared to the sham group.
Figure 3
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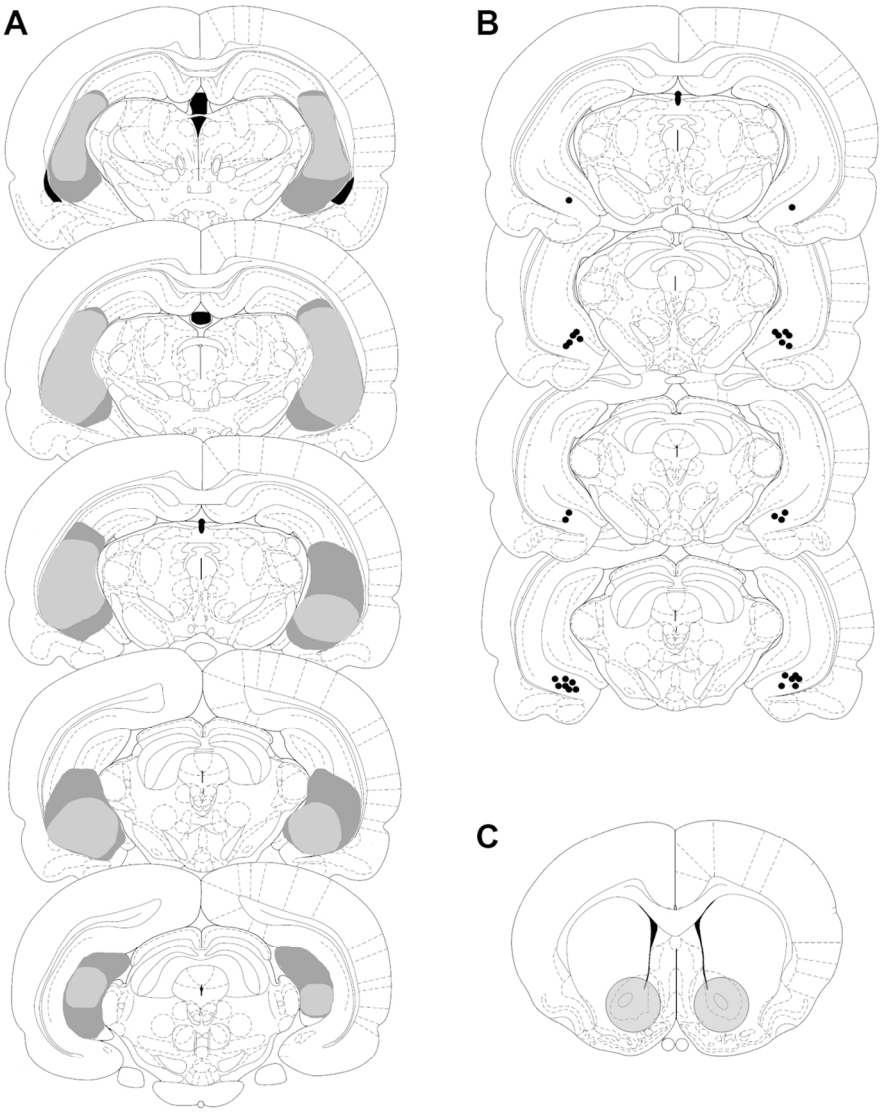

Figure 4. Schematic representation (adapted from Paxinos and Watson, 2007) of (A) NMDA-induced lesions of the ventral hippocampus (coronal sections presented from 4.52 to 6.30 mm posterior to bregma), (B) cannulas bilaterally targeted at the ventral subiculum (coronal sections presented from 5.30 to 6.30 mm posterior to bregma), and (C) tissue punches taken from the nucleus accumbens (coronal section presented at 1.20 anterior to bregma). For ventral hippocampal lesions, the largest (dark gray) and smallest (light gray) lesions were identified. For cannula placements, black circles represent the location of the infusion cannula tip. For tissue punches, the location of the 2-mm tissue punch is shaded (light gray).
Figure 4
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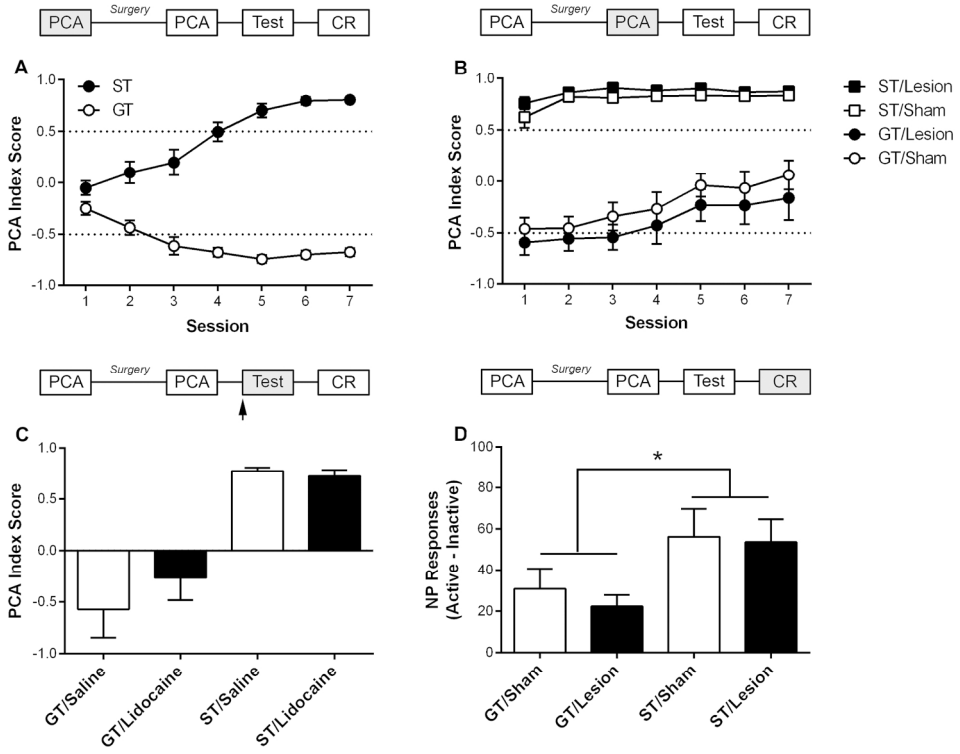

Figure 5. (A) Rats underwent seven daily sessions of Pavlovian conditioned approach (PCA) training, and the average PCA index scores of Sessions 6 and 7 were used to phenotype STs and GTs. (B) After performing surgeries to lesion the ventral hippocampus (Lesion) or implant guide cannulas targeted at the ventral subiculum (Sham), rats underwent another seven daily sessions of PCA training. (C) Next, sham rats were infused with either lidocaine (20% solution) or saline in a counter-balanced manner immediately before two additional PCA training sessions, which served as a temporary inactivation test. (D) Finally, all rats underwent a conditioned reinforcement test during which active but not inactive nose-poke (NP) responses resulted in 2-s presentations of the lever. Data are presented as mean ± S.E.M. * - $p < 0.05$.

Figure 5
176x140mm (300 x 300 DPI)

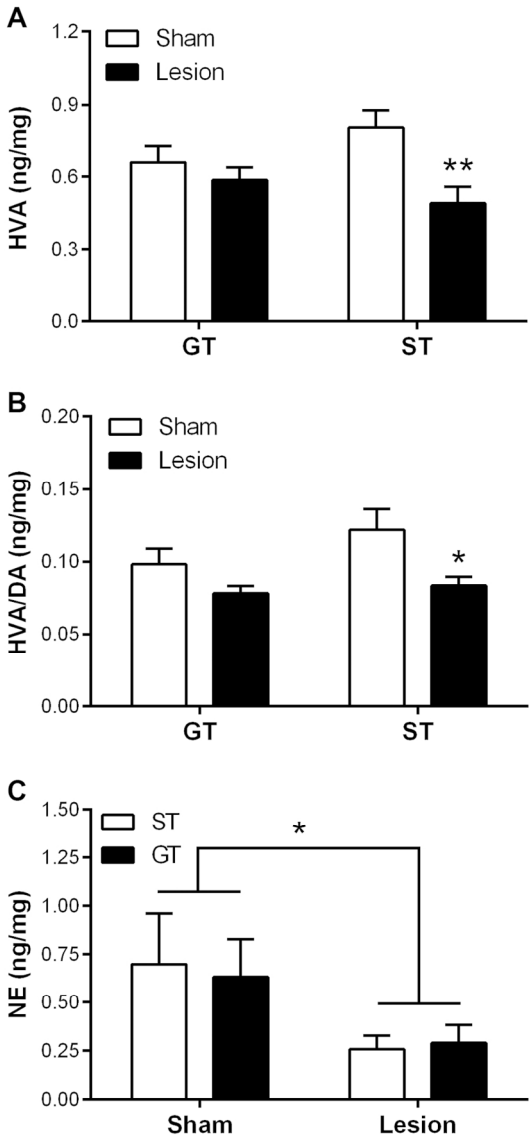

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Figure 6
85x172mm (300 x 300 DPI)