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The role of the laterodorsal tegmental nucleus in methamphetamine conditioned place preference and locomotor activity

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Abstract

Methamphetamine (METH) indirectly stimulates the laterodorsal tegmental nucleus (LDT) acetylcholine (ACh) neurons to increase ACh within the ventral tegmental area (VTA). LDT ACh inhibition attenuates METH and saline locomotor activity. The aim of these experiments was to determine whether LDT ACh contributes to METH conditioned place preference (CPP). C57BL/6J mice received a bilateral electrolytic or sham lesion of the LDT. After recovery, mice received alternating pairings of METH (0.5 mg/kg) and saline with distinct tactile floor cues over 8 days. During preference tests, mice were given access to both floor types and time spent on each was recorded. Mice were tested again after exposure to both extinction and reconditioning trials. Brains were then processed for choline acetyltransferase immunohistochemistry to label LDT ACh neurons. Lesioned mice had significantly fewer LDT ACh neurons and showed increased saline and METH locomotor activity during the first conditioning trial compared to sham mice. Locomotor activity (saline and METH) was negatively correlated with the number of LDT ACh neurons. Lesioned and sham mice showed similar METH CPP following conditioning, extinction and reconditioning trials. LDT ACh neurons are not necessary for METH reward as indexed by CPP, but may be important for basal and METH-induced locomotor activity.

Keywords

electrolytic lesion; methamphetamine; reward; locomotor activity; acetylcholine; inbred mice (C57BL/6)

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Methamphetamine (METH) is a highly addictive psychostimulant that induces dopamine (DA) release from terminals within the reward pathway independent of neuronal stimulation [1]. Recent evidence shows that METH selectively stimulates the LDT to increase acetylcholine (ACh) levels within the VTA [2, 3]. The major cholinergic input to the VTA originates in the LDT and the posterior portion of the pedunculopontine tegmental nucleus (PPT) [4–7]. Previous studies showed that electrical stimulation of the LDT, results in DA release within the mesocorticolimbic pathway via activation of cholinergic receptors within the VTA [8–10]. Furthermore, reversible inhibition of LDT ACh neurons attenuates basal and METH-induced locomotor activity in mice [3] and cocaine and food self-administration in rats [11]. The rewarding effects of drugs of abuse can be inferred from their ability to establish Pavlovian associations with environmental cues, enabling those cues to induce approach behavior in the absence of drug [12]. The goal of this experiment was to determine the role of the LDT cholinergic neurons in conditioned cue-induced drug seeking behavior.

Although we previously reversibly inhibited LDT cholinergic neurons using intra-LDT microinjections of the M2 subtype-preferring cholinergic agonist, oxotremorine, unpublished findings in our lab suggest that acquisition of conditioned place preference (CPP) in mice is impaired by the handling involved in repeated microinjections of vehicle solutions. Thus, we decided to first test the effects of pre-conditioning bilateral electrolytic LDT lesions on the acquisition of METH CPP; we confirmed cholinergic cell loss using choline acetyltransferase (ChAT) immunohistochemistry (IHC). We hypothesized that a bilateral LDT lesion would attenuate the acquisition of METH CPP.

A total of 48 male, C57BL/6J mice (9 weeks old at surgery) were used (Jackson Laboratory). All procedures were carried out in accordance with the National Research Council of the National Academies [13] and approved by the Institutional Animal Care and Use Committee at Oregon Health and Science University.

We stereotaxically (David Kopf Instruments, Tujunga, CA) targeted the LDT (AP: −5.02, ML: ± 0.65, DV: −3.5) [14] in anesthetized mice (n = 24/group) for bilateral electrolytic lesion (0.5 mA for 2 s) or sham lesion (electrode insertion only). We targeted the dorsomedial aspect of the LDT since this area is reported to be the most concentrated with putative ACh neurons in the rat [15] and also corresponds to the boundaries of the LDT in the mouse [14]. We chose to perform electrolytic lesions over excitotoxic because we had already identified the parameters (current x duration) that produced appropriately sized lesions. To characterize the extent of cholinergic cell loss, we performed ChAT IHC after the experiment. The LDT was sectioned through its entire anterior-posterior extent and two sections at each rostro-caudal level (−5.0, −5.2, and −5.4, relative to bregma) were selected to represent the LDT, for a total of 6 sections per mouse. Free-floating sections were quenched with 0.3% H_2O_2 in PBS and blocked using 4.5% normal Horse (Vector Labs) in PBS and 0.3% Triton-X 100 (Sigma-Aldrich). Sections were incubated overnight in the primary antibody directed at ChAT (1:5000, Millipore) in PBS/Triton with 0.1% bovine serum albumin. Detection of the primary antibody was accomplished with 0.5% biotinylated anti-goat secondary antibody (raised in horse; Vector Labs) in PBS/Triton-X. The immunoreaction was detected using a Vectastain ABC kit (Vector Labs) in PBS/Triton-X and developed with a DAB kit (Thermo Scientific). Sections were mounted on slides and

ChAT positive cells were counted manually with an Olympus BX51 microscope using QCapture (QImaging v2.8.1) and averaged for each level. For each subject, the average number of ChAT stained cells at each level was added together to create a total number of ChAT stained cells within the LDT.

Lesioned mice with less than 50% ChAT cell loss compared to sham mice were not included in activity or preference analyses, but were included in the correlational analyses (n=9). We also excluded some sham and lesioned mice due to poor ChAT staining or procedural errors. Final group sample sizes are listed in the figure legends. LDT-lesioned mice (Fig. 1a) included in the analyses had significantly fewer (66%) ChAT stained cells in the LDT than sham-operated mice (Fig. 1b) (63.5 ± 6.6 vs. 189.9 ± 16.9, respectively; $t_{26} = 5.8$, $p < 0.0001$).

All mice were exposed to an unbiased two-compartment place conditioning procedure using an unbiased apparatus equipped with infrared photobeams described previously [16]. On the first day (habituation), mice received IP saline (10 ml/kg) and were immediately placed into the apparatus on a paper floor for 5 min. Mice were randomly assigned to receive METH (0.5 mg/kg, NIDA drug supply program, Research Triangle Park, NC) paired with one of two floor types: grid or hole. In the GRID+ subgroup (n= 12/subgroup), METH was injected immediately before placement on the grid floor (CS+) whereas saline was injected before placement on the hole floor (CS−). These contingencies were reversed for mice in the GRID − conditioning subgroup. Mice received one 30-min trial per day across 8 days for a total of four trials of each type. Order of exposure to METH and saline was counterbalanced. Two preference tests were administered: one after the first two trials of each type (2 METH and 2 saline), and one after all four trials (4 METH and 4 saline). This design allowed us to evaluate the effect of LDT lesion on a weak (test 1) or strong (test 2) METH CPP. Mice received IP saline and were placed in the center of the apparatus with access to both the CS+ and CS− floors during each 30 min test. Activity was measured as consecutive beam breaks and preference was measured by calculating the time spent on each floor type.

Preference was expressed as the time spent on the grid floor for subjects that had METH paired with the grid (GRID+) or hole (GRID−) floor. A significant difference between these subgroups provides evidence of place conditioning [16]. Grid time data were analyzed by factorial ANOVA using conditioning subgroup (GRID+ or GRID−) and surgical group (Sham or Lesion) as between subjects factors and test as a repeated measure. Activity data were also analyzed by factorial ANOVA using trials and trial type as repeated measures; violations to sphericity were corrected using Greenhouse-Geisser.

Figure 2 shows that sham-operated and LDT lesioned mice had significant but similar levels of METH CPP during the first (2a) and second (2b) post-conditioning tests (main effect of conditioning: $F_{1,30} = 113.2$, $p < 0.0001$). Moreover, preference increased between tests (test x conditioning interaction: $F_{1,30} = 13.2$, $p = 0.001$). There were no interactions of surgical group with conditioning or test, but there was a main effect of surgical group ($F_{1,30} = 8.8$, $p < 0.05$) that reflected slightly more time (~2.1 s) spent on the grid floor by sham mice, regardless of conditioning subgroup. Sham (63.9 ± 2.5) and lesioned (59.8 ± 3.7) mice exhibited similar levels of test activity.

Three-way repeated measures ANOVA of activity during conditioning (Fig. 3a) yielded significant two-way interactions of trial x surgical group ($F_{1.65,30} = 6.59$, $p = 0.005$) and trial x trial type ($F_{2.57,30} = 6.28$, $p = 0.001$). There were also significant main effects of trial ($F_{1.65,52.7} = 10.2$, $p < 0.0005$) and trial type ($F_{1,32} = 81.66$, $p < 0.0005$), and a trend for a main effect of surgical group ($F_{1,32} = 4.15$, $p = 0.05$). Follow-up analysis of the trial x surgical group interaction (collapsed across trial type) showed that lesioned mice were significantly more active than sham mice on the first trial ($F_{1,32} = 15.3$, $p < 0.0005$). Bonferroni-corrected posthoc analysis of the trial x trial type interaction (collapsed across surgical group) found that METH significantly increased activity on all four trials (p's < 0.02).

We tested whether a LDT lesion would attenuate the extinction or reconditioning of METH CPP because a previous study showed that although a post-conditioning PPT lesion had no effect on the extinction of morphine CPP, lesioned rats failed to reacquire morphine place preference [17]. The same mice that had acquired METH CPP were exposed to extinction followed by reconditioning with additional preference tests after each phase.

During the extinction phase, mice received two 30-min trials (CS+ and CS−) every day, separated by 4 h, for 6 days in the same counterbalanced order used during conditioning. Mice received a saline injection immediately before each trial. Preference tests were administered after days 2, 4 and 6 of extinction in order to monitor the development of extinction. The magnitude of METH preference assessed following extinction was significantly reduced compared to the second post-conditioning test (test x conditioning interaction: $F_{1,29} = 14.73$, $p = 0.001$), with sham and lesion mice showing a similar reduction in METH preference after extinction (main effect of conditioning; $F_{1,29} = 23.65$, $p < 0.0001$) (Fig. 2c). As during the post-conditioning tests, sham mice spent slightly more time on the grid floor across both the GRID+ and GRID− subgroups compared to lesioned mice ($F_{1,29} = 11.24$, $p = 0.002$). There was no lesion effect on test activity (lesion, 71.5 ± 7.3; sham, 66.0 ± 3.1).

A three-way repeated measures ANOVA of activity during extinction showed a main effect of trial ($F_{3.3, 26} = 7.55$, $p < 0.0001$), and a significant trial x surgical group interaction ($F_{3.3, 26} = 2.79$, $p = 0.039$ (Fig. 3b). Bonferroni corrected post hoc analyses (collapsed across trial type), however, found no significant group differences on individual trials. Sham mice were more active on the first trial compared to trials 2, 3, 4, 5 and 6 (all p's < 0.01) and lesioned mice were more active on trials 1 and 2 compared to trials 2 and 5, respectively (all p's < 0.05).

Reconditioning began the day after the post-extinction test. Mice received one CS+ and one CS− trial (30 min) over 2 days as during conditioning. A preference test administered the day after the last reconditioning trial revealed that lesioned and sham-operated mice exhibited an increase in METH preference compared to the post-extinction preference test (test x conditioning interaction: $F_{1,29} = 29.6$, p = 0.023) (Fig. 2d). Sham and lesion mice showed a similar level of METH preference after reconditioning (main effect of conditioning; $F_{1,29} = 104.45$, $p < 0.0001$), with sham mice spending slightly more time on the gird floor across both conditioning subgroups compared to lesioned mice ($F_{1,29} = 12.56$,

$p = 0.001$). There was no lesion effect on test activity (sham: 73.77 ± 3.56; lesion: 75.52 ± 7.87). Figure 3c shows that sham and lesioned mice showed significant locomotor activation to 0.5 mg/kg METH during the reconditioning trial (main effect of trial type; $F_{1,31} = 45.74$, $p < 0.0001$).

In order to further investigate the relationship between LDT cholinergic cells and METH locomotor activity and reward, we performed Pearson correlations between the number of LDT ChAT cells and locomotor activity or CPP (using percent time on the METH paired floor during each test). The number of LDT ChAT cells was negatively correlated with locomotor activity on the habituation day ($r = -0.60$, $p < 0.0001$, N = 37) and on conditioning trial 1 following METH ($r = -0.38$, $p = 0.019$, N = 37) or saline ($r = -0.51$, $p = 0.001$, N = 37). The number of LDT ChAT cells was not correlated with locomotor activity during conditioning trials 2–4 (METH or saline). Finally, the number of LDT ChAT cells was not correlated with activity during reconditioning or extinction trials or with percent time on the METH paired floor for any of the preference tests.

These data show that a bilateral LDT lesion has no effect on the acquisition, expression, extinction or reconditioning of METH CPP. Moreover, although METH can increase DA levels independently of neuronal stimulation, the data indicate that the LDT is not necessary for METH-conditioned cues to elicit drug seeking in the absence of METH (i.e., during preference tests). Furthermore, cholinergic cell loss, measured by ChAT immunohistochemistry, was not correlated with METH preference following conditioning, extinction or reconditioning. However, the number of LDT cholinergic cells was negatively correlated with METH and saline locomotor activity during habituation and conditioning trial 1. These negative correlations suggest that the LDT, and possibly ACh in particular, are important for mediating locomotor activity.

Although LDT ACh does not appear important for conditioned METH reward, a previous study showed that reversible inhibition of LDT cholinergic neurons attenuated cocaine and food self-administration in rats [11]. While this study reported that intra-LDT OXO microinjection marginally enhanced locomotor activity in food trained (i.e., cocaine naïve) rats, it did not assess whether intra-LDT OXO affected cocaine-induced activity [11]. It is possible that, in combination with cocaine, intra-LDT OXO disrupted locomotor activity and thereby attenuated cocaine self-administration.

We previously found that administration of muscarinic cholinergic agonists into the LDT attenuates VTA acetylcholine levels and locomotor activity [3]. Furthermore, excitotoxic lesion of the LDT in rat also inhibited locomotor activity [18, 19]. Conversely, IP, intra-PPT or intra-LDT administration of the muscarinic ACh antagonist scopolamine induced locomotor activation, which the authors hypothesized occurred via disinhibtion of mesopontine cholinergic projection neurons to VTA DA neurons [18, 20]. The role of the cholinergic system in psychostimulant-induced locomotor activation has also been investigated. Scopolamine pretreatment increased acute cocaine-induced locomotor activity and time spent in stereotypy [21]. Moreover, scopolamine administered during cocaine CPP trials blocked conditioned hyperlocomotion [22]. Finally, IP scopolamine or trihexyphenidyl

(a muscarinic ACh receptor antagonist) enhanced METH and cocaine locomotor activity [23].

Previously, we showed that LDT ACh neurons are important for METH-induced increases in VTA ACh levels and locomotor activity, but not NAc DA levels [3]. While the current experiment indicates that the LDT is not necessary for the expression of a conditioned METH place preference, LDT ACh neurons may be important for locomotor activity following IP METH or saline. We hypothesize that METH acts via the mesopontine cholinergic projections to the VTA to affect locomotor activity, but these cholinergic neurons are not required for METH-induced DA responses or for cue-elicited METH seeking.

Although the LDT lesion did not enhance locomotor activity across all METH conditioning trials, we previously observed a more long-lasting effect of LDT lesion on low-dose METH-induced locomotion (0.25 mg/kg, unpublished observations). This suggests that the 0.5 mg/kg methamphetamine dose used in the current experiment may have masked an effect of the LDT to modulate locomotor activity. Reversible inhibition of LDT cholinergic neurons significantly inhibits saline and METH activity [3]; however, the current results showed that a lesion of the entire LDT induced a general locomotor activation. This discrepancy may suggest that other neurotransmitters in the LDT or the PPT are involved in the balance of cholinergic locomotor activity. The GABA and glutamate projection neurons in the LDT were likely also significantly reduced as a result of the electrolytic lesion used to destroy the LDT in the current study. Previous research suggests that $GABA_A$ receptors are involved in amphetamine sensitization [24] and that $GABA_B$ receptors in the VTA are involved in acute ethanol locomotor activation [25]. In addition, plasticity of the LDT-to-VTA glutamate projections is involved in amphetamine sensitization [26]. We also cannot exclude the possibility that fibers of passage in the LDT were damaged following the electrolytic lesion. Thus, nuclei from which these fibers originate could have also been damaged and contributed to the potentiation of locomotor activity. Additionally, the bilateral LDT lesion may also have triggered a compensatory response from cholinergic neurons in the PPT, resulting in stronger connections to the VTA. Enhanced PPT to VTA projections may also account for the difference in locomotor activity between our previous study, which used acute, reversible ACh inhibition, and the current findings. In order to fully elucidate the role of LDT ACh in basal and psychostimulant-induced locomotor behavior, future studies could employ, for instance, an optogenetic approach to selectively activate or inhibit LDT-to-VTA ACh projection neurons.

In combination with our previous findings and the current literature regarding the role of LDT ACh in activity and reward, we conclude that the cholinergic neurons in the LDT are not necessary for the expression of a conditioned cue-elicited drug seeking behavior in the absence of METH's DA-potentiating effects, but may be involved in METH and saline locomotor activity.

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References

1. Sulzer D, Sonders MS, Poulsen NW, Galli A. Mechanisms of neurotransmitter release by amphetamines: a review. Progress in neurobiology. 2005; 75:406–33. [PubMed: 15955613]
2. Dobbs LK, Mark GP. Comparison of systemic and local methamphetamine treatment on acetylcholine and dopamine levels in the ventral tegmental area in the mouse. Neuroscience. 2008; 156:700–11. [PubMed: 18760336]
3. Dobbs LK, Mark GP. Acetylcholine from the mesopontine tegmental nuclei differentially affects methamphetamine induced locomotor activity and neurotransmitter levels in the mesolimbic pathway. Behavioural brain research. 2012; 226:224–34. [PubMed: 21945297]
4. Bolam JP, Francis CM, Henderson Z. Cholinergic input to dopaminergic neurons in the substantia nigra: a double immunocytochemical study. Neuroscience. 1991; 41:483–94. [PubMed: 1678502]
5. Geisler S, Zahm DS. Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. The Journal of comparative neurology. 2005; 490:270–94. [PubMed: 16082674]
6. Oakman SA, Faris PL, Kerr PE, Cozzari C, Hartman BK. Distribution of pontomesencephalic cholinergic neurons projecting to substantia nigra differs significantly from those projecting to ventral tegmental area. Journal of Neuroscience. 1995; 15:5859–69. [PubMed: 7666171]
7. Omelchenko N, Sesack SR. Laterodorsal tegmental projections to identified cell populations in the rat ventral tegmental area. The Journal of comparative neurology. 2005; 483:217–35. [PubMed: 15678476]
8. Forster GL, Blaha CD. Laterodorsal tegmental stimulation elicits dopamine efflux in the rat nucleus accumbens by activation of acetylcholine and glutamate receptors in the ventral tegmental area. Eur J Neurosci. 2000; 12:3596–604. [PubMed: 11029630]
9. Forster GL, Yeomans JS, Takeuchi J, Blaha CD. M5 muscarinic receptors are required for prolonged accumbal dopamine release after electrical stimulation of the pons in mice. Journal of Neuroscience. 2001; 21 RC 190 (1–6).
10. Lodge DJ, Grace AA. The laterodorsal tegmentum is essential for burst firing of ventral tegmental area dopamine neurons. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103:5167–72. [PubMed: 16549786]
11. Shabani S, Foster R, Gubner N, Phillips TJ, Mark GP. Muscarinic type 2 receptors in the lateral dorsal tegmental area modulate cocaine and food seeking behavior in rats. Neuroscience. 2010; 170:559–69. [PubMed: 20667466]
12. Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology. 2000; 153:31–43. [PubMed: 11255927]
13. Academies NRCotN. Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. 2003.
14. Paxinos, G.; Franklin, KBJ. The mouse brain in stereotaxic coordinates. 2. San Diego, CA: Academic Press; 2001.
15. Wang HL, Morales M. Pedunculopontine and laterodorsal tegmental nuclei contain distinct populations of cholinergic, glutamatergic and GABAergic neurons in the rat. Eur J Neurosci. 2009; 29:340–58. [PubMed: 19200238]
16. Cunningham CL, Ferree NK, Howard MA. Apparatus bias and place conditioning with ethanol in mice. Psychopharmacology. 2003; 170:409–22. [PubMed: 12955296]
17. Bechara A, van der Kooy D. The tegmental pedunculopontine nucleus: a brain-stem output of the limbic system critical for the conditioned place preferences produced by morphine and amphetamine. J Neurosci. 1989; 9:3400–9. [PubMed: 2795130]
18. Laviolette SR, Priebe RP, Yeomans JS. Role of the laterodorsal tegmental nucleus in scopolamine- and amphetamine-induced locomotion and stereotypy. Pharmacol Biochem Behav. 2000; 65:163–74. [PubMed: 10638650]

19. Alderson HL, Latimer MP, Winn P. Involvement of the laterodorsal tegmental nucleus in the locomotor response to repeated nicotine administration. Neuroscience letters. 2005; 380:335–9. [PubMed: 15862913]

20. Mathur A, Shandarin A, LaViolette SR, Parker J, Yeomans JS. Locomotion and stereotypy induced by scopolamine: contributions of muscarinic receptors near the pedunculopontine tegmental nucleus. Brain Res. 1997; 775:144–55. [PubMed: 9439838]

21. Heidbreder CA, Shippenberg TS. Evidence for an involvement of muscarinic cholinergic systems in the induction but not expression of behavioral sensitization to cocaine. Synapse (New York, NY. 1996; 24:182–92.

22. Itzhak Y, Martin JL. Scopolamine inhibits cocaine-conditioned but not unconditioned stimulant effects in mice. Psychopharmacology. 2000; 152:216–23. [PubMed: 11057526]

23. Shimosato K, Nagao N, Watanabe S, Kitayama S. Suppressive effects of trihexyphenidyl on methamphetamine-induced dopamine release as measured by in vivo microdialysis. Synapse (New York, NY. 2003; 49:47–54.

24. Panhelainen AE, Vekovischeva OY, Aitta-Aho T, Rasanen I, Ojanpera I, Korpi ER. Diazepam-induced neuronal plasticity attenuates locomotor responses to morphine and amphetamine challenges in mice. Neuroscience. 2011; 192:312–21. [PubMed: 21782898]

25. Boehm SL 2nd, Piercy MM, Bergstrom HC, Phillips TJ. Ventral tegmental area region governs GABA(B) receptor modulation of ethanol-stimulated activity in mice. Neuroscience. 2002; 115:185–200. [PubMed: 12401333]

26. Nelson CL, Wetter JB, Milovanovic M, Wolf ME. The laterodorsal tegmentum contributes to behavioral sensitization to amphetamine. Neuroscience. 2007; 146:41–9. [PubMed: 17321058]

Highlights

- We test if laterodorsal tegmental nucleus acetylcholine is involved in METH reward.
- We bilaterally lesioned the laterodorsal tegmental nucleus in mice.
- Lesion did not alter acquisition, extinction or reinstatement of METH preference.
- Mice with lesion were more active following saline or METH injection.
- Number of acetylcholine neurons was negatively correlated with locomotor activity.

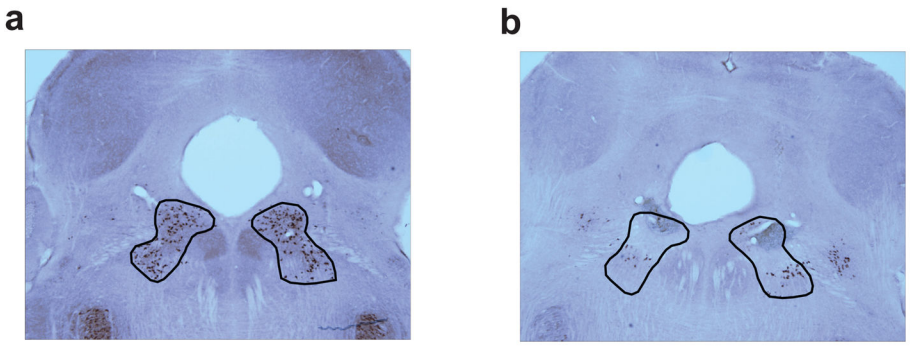

Fig. 1. ChAT IHC in the LDT of lesioned and sham operated mice

A representative section (4x) of a LDT-lesioned (a) and sham-operated (b) subject illustrates the reduction in the number of choline acetyltransferase (ChAT) labeled cells following a bilateral LDT lesion. The boundaries of the LDT are outlined in black.

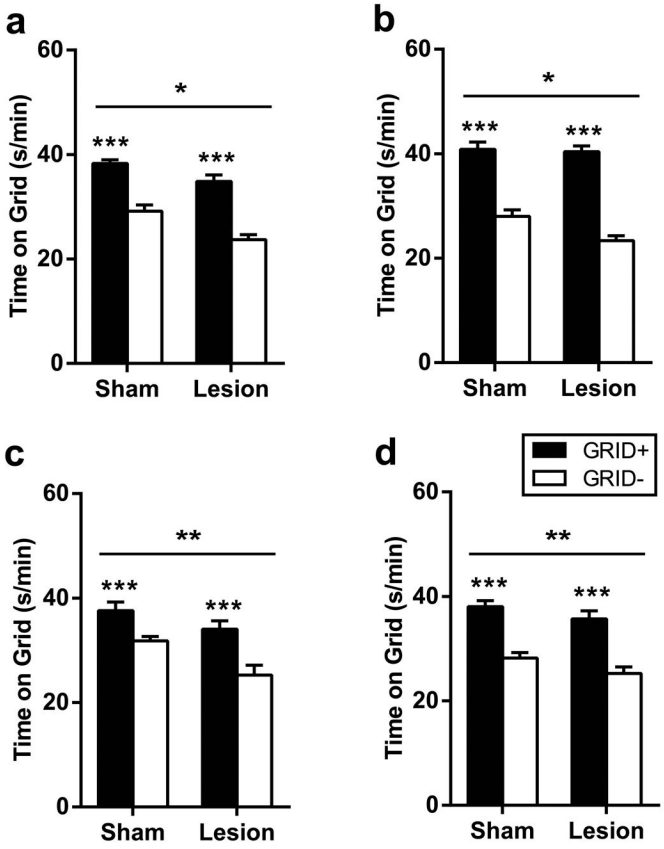

Fig. 2. LDT lesion has no effect on cocaine conditioned place preference

The magnitude of METH conditioned place preference after four (a) and eight (b) conditioning trials, extinction (c), and reconditioning (d) is shown for subjects that had METH paired with the grid floor (GRID+; black bars) or hole floor (GRID−; white bars). Sham-operated (n = 23, panels a and b; n = 22, panels c and d) and LDT lesioned (n = 11) subjects showed similar METH place preference following conditioning, extinction and reconditioning trials (*** $p < 0.0001$ GRID+ vs. GRID−). Sham mice spent more time on the grid floor than lesioned mice regardless of conditioning subgroup during the post-conditioning (* $p < 0.05$) and post-extinction tests (** $p < 0.01$).

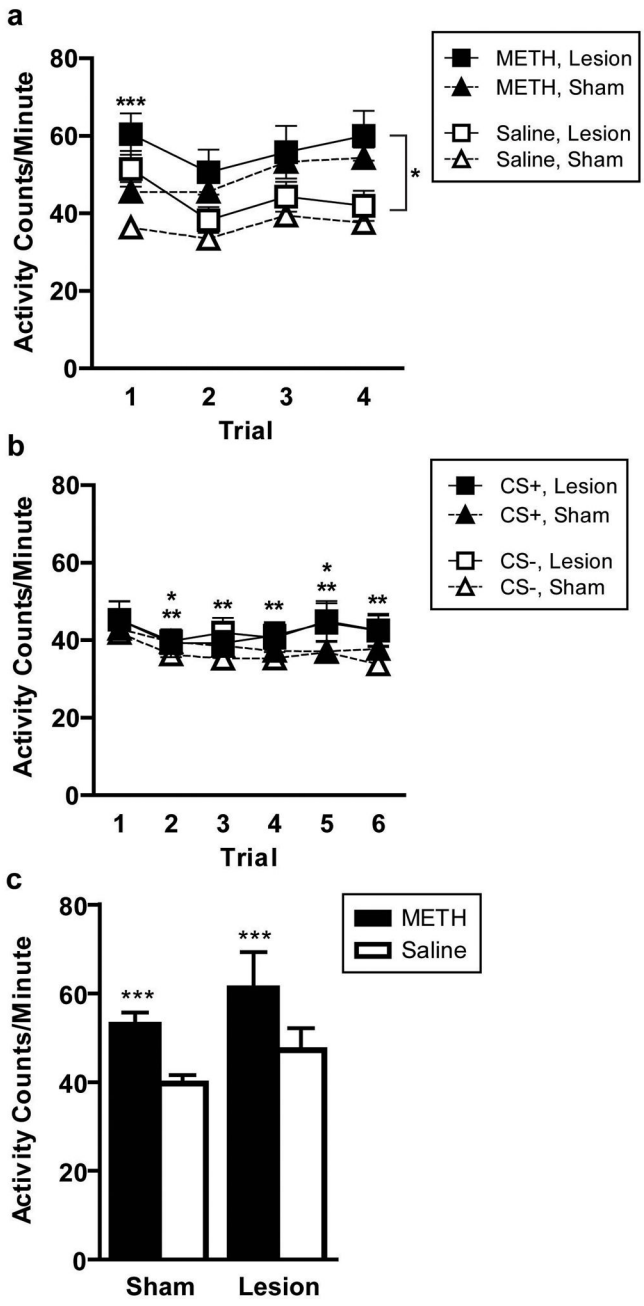

Fig. 3. LDT lesioned mice show enhanced locomotor activity compared to sham operated controls

Locomotor activity following METH (CS+, black symbols) and saline (CS−, white symbols) is shown for the conditioning (a), extinction (b), and reconditioning trials (c). During conditioning trial 1, lesioned subjects (n = 11; squares) were more active than sham subjects (n = 23; triangles) following saline or METH (*** $p < 0.001$). Additionally, across all conditioning trials, sham and lesioned subjects were more active following IP METH compared to saline (* $p < 0.05$). During extinction trials, sham subjects (n = 22) were more active on trial 1 than trials 2, 3, 4, 5 and 6 (** $p < 0.01$), and lesioned subjects were more

active on trials 1 and 2 than trials 2 and 5, respectively (* $p < 0.05$). During the reconditioning trials, IP METH increased locomotor activity in sham (n = 22) and lesioned subjects (n = 11) compared to saline (*** $p < 0.0001$).