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Short communication

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

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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.

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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/6 J 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.

1. Introduction

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

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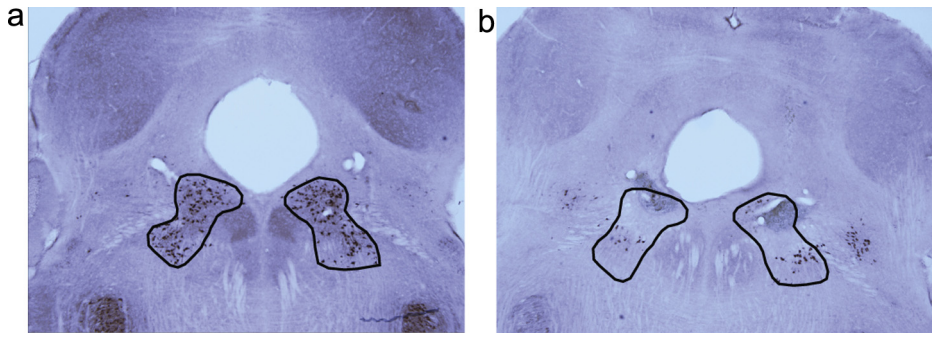

Fig. 1. A representative section (4×) of a LDT-lesioned (a) and sham-operated (b) subject illustrates the reduction in the number of choline acetyltransferase (ChAT) labeled cells following a bilateral LDT lesion. The boundaries of the LDT are outlined in black.

(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 × 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 serum (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.

Fig. 2 shows that sham-operated and LDT lesioned mice had significant but similar levels of METH CPP during the first (a) and second (b) post-conditioning tests (main effect of conditioning: $F_{1,30}$ = 113.2, $p \leq 0.0001$). Moreover, preference increased between tests (test × 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 × surgical group ($F_{1.65,30}$ = 6.59, p = 0.005) and trial × trial type ($F_{2.57,30}$ = 6.28, p = 0.001). There were also significant main effects