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Supplementary Information for

Spatial contextual recognition memory updating is modulated by dopamine release in the dorsal hippocampus from the locus coeruleus

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Materials and Methods

1. Subjects

We employed C57BL/6J wild type (WT) and TH-Cre mice (FI12 line; 3 - 4 months old / 22 - 26 g) expressing Cre-recombinase protein under the control of the endogenous tyrosine TH promoter. Dr. Rui Costa from Champalimaud Center kindly donated TH-Cre breeder mice. All animals were individually housed at 23º C with water and food *ad libitum* and were kept under a 12-hour light/dark cycle. All study subjects were randomly assigned to these groups. According to the Mexican regulations (NOM-062-ZOO-1999), all procedures followed the Guidelines for the Care and Use of Laboratory Animals, National Institutes of Health. The Committee for Animal Care and Use of Cellular Physiology Institute approved the procedures (FBR125-18).

1.1 Genotype

All TH-Cre mice were genotyped by the Hotshot method, previously reported (51). The tail snipping procedure was performed; we removed 1 mm of the tail mice. We lysed the tail snip in an alkaline reagent (25 mM NaOH, 0.2 mM disodium EDTA) under heat at 95 ºC for 1 hour for DNA extraction. After, neutralization buffer was added (1 M Tris-HCl, pH 7.4) and centrifuged (2500 rpm, 2 minutes, Hermle Z 233 MK-2). Finally, DNA supernatant was recovered and stored. The DNA was amplified with PCR (201443, QIAGEN). Primers sequences: Cre forward primer 5'-AGC CTG TTT TGC ACG TTC ACC-3'; Cre reverse primer 5'-GGT TTC CCG CAG AAC CTG AA-3' (Sigma-Aldrich, USA).

2. Viral vectors

Viral vectors Cre-inducible adeno-associated viruses (AAV) were obtained from the University of North Carolina, Gene Therapy Center Vector Core (USA). The viral concentration was $4.0x10^{12}$ particles per ml for AAV5/EF1a-DIO-eNpHR3.0-eYFP (eNpHR), $6.0x10^{12}$ particles per ml for AAV5/EF1a-DIO-eYFP (eYFP). Viral vectors were stored in aliquots at -80ºC.

3. Stereotaxic surgery

Regardless of the group they belonged to, all animals were first anesthetized using a blend of oxygen (1L per min) and isoflurane (induction 5%; Maintenance 1-2%; Vip 3000 matrix) and placed into a stereotaxic apparatus (RWDM life science, USA). WT and TH-Cre mice were manipulated with pharmacology and optogenetic techniques, respectively. The coordinates for all surgical procedures were taken from the Allen Brain Atlas (Allen Institute for Brain Science) (95). For pharmacology experiments, the cannulae (0.6 mm, C316G-24, Plastics One, USA) were aimed at the dorsal hippocampus (−2.1 mm AP; ±1.5 mm ML; −0.25 mm DV to bregma).

To perform the optogenetic experiments, the TH-Cre mice received a bilateral injection containing either the virus eNpHR or eYFP (0.5 µL per hemisphere) into the VTA (−3.0 mm AP; ±0.6 mm ML; −5.5 mm DV to bregma) or the LC (−5.5 mm AP; ±0.9 mm ML; −3.3 mm DV to bregma). We used a calibrated glass micropipette (5µL, Drummond, USA). Animals assigned to OLM and OLM/Microdialysis groups were implanted with optical fibers (0.22 NA, 200 µm diameter; Doric Lenses, Canada) into the dorsal hippocampus CA1 (−2.40 mm AP; ±2.0 mm ML; −1.00 mm DV to bregma). Finally, OLM/Microdialysis subjects were unilaterally implanted with a cannula guide (CMA/7; CMA Microdialysis, Sweden) into the hippocampus CA1 (−3.0 mm AP; ±2.0 mm ML; −1.5 mm DV to bregma) with a 25º frontal angle. TH-Cre animals were allowed three weeks and WT one week for their recovery.

4. Object Location Memory (OLM)

The OLM was evaluated in a 33 x 33 x 30 cm wooden box. A black and white striped contextual cue (5 x 30 cm, with 1cm stripes) was glued to one of the walls. The stimulus objects were two Lego figures fixed with Velcro to the arena's corners (6.5 cm from the walls). The role of familiar localization (FL) or novel localization (NL) and their relative positions were counterbalanced. The box was placed in a dim-light room. All animals completed this task except those assigned to the electrophysiology group.

Animals were habituated to the experimental arena for three consecutive days, allowing them to explore the arena without stimulus objects (Habituation). In two training sessions, mice were introduced into the arena containing the same object configuration (Training). Exploration was considered every time the mouse sniffed or touched one object with the forepaws. In contrast, exploration was not considered when the mice were sitting or turning around. Twenty-four hours after training, one update session was conducted in which animals were reintroduced into the arena with one object placed in the original position and the other in a novel position (UPDATE). The following day, the last test session was completed using the same object configuration of the update session to measure memory updating (TEST) (24).

The duration of all sessions was 10 minutes. They were carried out at the same time of the day, and the task was performed one mouse at a time. Training, update, and test sessions were recorded and further analyzed using the Debut Video Capture computer software (NCH software, USA) to assess the time every animal spent exploring each object.

4.1 Drug administration

The WT mice were divided into three groups depending on the drug injected: a) DA D1/D5 receptor antagonist R (+)-SCH-23390 hydrochloride (SCH) (2.5µg, Sigma, St. Louis, MO, USA). b) Beta-receptor antagonist (-)-Propranolol hydrochloride (PROP) (5µg, Sigma, St. Louis, MO, USA). c) Isotonic Saline Solution 0.9% (ISS). Each group was further divided based on whether they received the injection: before 30 minutes with propranolol and before 15 min for SCH and ISS, as previously reported (39, 44, 46) or immediately after the update session. The infusion needle was connected via polyethylene tubing to a 10µl Hamilton micro-syringe (The Hamilton Company Quality System, ISO 9001-2008, USA) driven by a micro-infusion pump (Cole Parmer Instruments). In all cases, injections were performed over 1 minute, and the injector was left for an additional minute to allow complete drug diffusion. The injected volume was 0.5 µl per hemisphere.

4.2 *Optogenetic manipulations*

The TH-Cre mice received light during or after the update session of the photoinhibition experiments. Light delivery comprised of a continuous pulse of 532 nm (150 mW; OEM Laser Systems, USA) on the fiber optic tip coupled with a patch cord (0.22 NA, diameter 62,5 µm, Thorlabs, USA) and was measured at 10-15 mW (51, 96–98).

4.3 Microdialysis

A CMA/7 membrane (CMA Microdialysis, Sweden) was inserted into a guide cannula previously implanted in mice belonging to the OLM/Microdialysis group. Ringer solution (MgCl2 12mM, NaCl 1.44 M, CaCl2 17 mM and KCl 48 mM) was perfused at a 0.25 µl/min rate using a micro-infusion continuous pump (100 pump CMA Microdialysis, Sweden).

Mice were placed into the arena in the absence of objects, and they were infused with Ringer solution to collect the neurotransmitter using the EICOM piping system (Concise Freely Moving System, EICOM, USA). Once the 30 minutes stabilization period concluded, three 16 minutes samples were collected to calculate the baseline. These samples were placed into a vial containing an antioxidant blend (Ascorbic acid 25 mM, Na_2EDTA 27 mM, and acetic acid 1 M).

Subsequently, a fourth fraction was collected while the animal conducted the update session and when it received the optogenetic light delivery. All samples were stored at -80ºC

Neurotransmitter analysis: capillary electrophoresis quantified neurotransmitter concentration (99). Briefly, all microdialysis samples were submitted to derivatization by adding 6μL of 3-(2-furoyl)-quinoline-2-carboxaldehyde (FQ, 16.67 mM, Molecular Probes; Invitrogen, USA). This reaction is catalyzed by 2μL KCN (24.5 mM) in borate buffer (10 mM pH 9.2) in the presence of 1 μL of an internal standard (0.075 mM, O-methyl-L-threonine; Fluka, USA). The mixture was incubated in the dark for 15 min at 65ºC. Subsequently, the neurotransmitters were detected with laser-induced fluorescence within a capillary electrophoresis system (P/ACE MDQ, Beckman Coulter; Pasadena, USA). Compound separation was based on a micelle electrokinetic chromatography method. Samples were hydro-dynamically injected into the capillary system at 0.5 psi for 5s. Separation occurred in the presence of a buffer (borates 35 mM, sodium dodecyl sulfate 25 mM, and 13% methanol HPLC grade, pH 9.6) at 20 kV. Neurotransmitters migrate until they are separated and detected by fluorescence using a LIF device (488nm). Signals were depicted as electropherograms that later were analyzed using 32Karat TM8.0 software (Beckman Coulter, USA). Neurotransmitters were identified by comparison with the standard electropherogram pattern (DA and NA standards). Signals were quantified by comparing the respective area under the curve with the internal standards.

5. Electrophysiology

The electrophysiology group mice infected with eNpHR or eYFP (described in the S*tereotaxic Surgeries* section) were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg) and placed in the stereotaxic apparatus. Subsequently, four electrodes made of stainless-steel wire (0.1 mm diameter coated with Teflon, Biomedical Instruments, Germany) were implanted. A monopolar recording electrode was placed in the hippocampus CA1 (−2.20 mm AP; +1.4 mm ML, -1.0mm to −1.50 mm DV). The second electrode, a bipolar stimulating one, was placed in the hippocampus CA3 (−1.50 mm AP; -2.0 mm ML, -1.0mm to −1.50 mm DV). Finally, the reference and ground electrodes were placed in the cortex (0.0 mm AP, -1.5 mm ML, 0.0 mm DV).

To measure the synaptic activity in the CA1, we analyzed the field excitatory postsynaptic potential slope (fEPSP). Basal responses were evoked by stimulation at a low frequency (0.25 Hz) with single biphasic square wave pulses of 0.25 ms duration every half-wave. Different stimulation intensities were evaluated to identify the maximum fEPSP slope. Baseline potentials, evoked to 50% of the maximum fEPSP slope, were recorded every 4 s for 15 min to ensure a representative baseline (fEPSP slope ±10%). LTP was induced by high-frequency stimulation of the Shaffer collaterals (HFS, three trains of 100 pulses at 100 Hz with 20 s inter train). After recording for one hour, all data was digitalized and stored using Datawave software (Sciworks, USA).

6. Histology

After submitting to the above procedures, all mice received a sodium pentobarbital overdose (75 mg/kg). Mice were transcardially perfused with saline solution and fixed with 4% paraformaldehyde phosphate buffer solution (PBS). Brains were removed, placed in a 4% paraformaldehyde solution for 48 h, and cryoprotected in 30% sucrose in PBS for another two days. Brains were then sectioned in 40 μm coronal slices with a cryostat (Leica Biosystem, USA) and stored in a trizma-base saline buffer (TBS, 0.1 M, pH 7.4).

Nissl staining was used to verify the location of the electrodes or cannula within the brains of the animals from the electrophysiology and pharmacology groups, respectively. Photomicrographs were taken with a Leica microscope (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). For immunofluorescence histology, tissue sections were incubated overnight with rabbit polyclonal anti-TH primary antibody (1:1000, Pel-Freez, USA) with 0.1% triton and 5% bovine

serum albumin (BSA) in TBS buffer (0.1 M, pH 7.4). After washing in TBS buffer, sections were incubated in goat anti-rabbit IgG secondary antibody conjugated with Cy3 (1:500, Millipore, Darm-Stadt, Germany) with 5% BSA in TBS buffer for two hours. Sections were incubated with DAPI (1μg/mL, for 10 min) and finally mounted in the Dako fluorescence medium. Images were taken using a confocal microscope (ZEISS LSM 800, Germany).

6.1. Nuclei and axons quantification

A quantitative analysis was performed to measure the eYFP positive nuclei and TH terminals in the hippocampus, CA1 region from the LC or the VTA. We choose the hippocampus CA1 region (-2.5 to -1.5 AP), VTA (-3.5 to -2.5 AP), and LC (-5.8 to -5.3 AP) (95). We process five tissue sections for the anti-TH immunofluorescence protocol previously described. First, in the serial sections of the hippocampus CA1, LC or VTA were captured with a 20X magnification on the Z-axis using a confocal microscope (ZEISS LSM 800, Germany). For nuclei analysis of VTA and LC, we process images with an automated protocol for TH+ and eYFP in Image J software (Auto-Threshold "Triangle dark"; (14, 255), "BlackBackground"). For TH terminals in the hippocampus CA1 from LC or VTA, we process images with automated protocol in Image J for eYFP (Auto-Threshold "Triangle dark"; (20, 255), "BlackBackground").

7. Statistics

Statistics analysis was conducted using GraphPad Prism software (version 7.00, USA). All graphs showed mean ± sem with a statistical significance of $p<0.05$. For immunohistochemistry, images were processed using the automated image J software. For area percent of nuclei and axons were compared with an unpaired t-student with two tails. The recognition index values between familiar and novel objects obtained during the OLM test were compared with mixed - ANOVA (between-subjects = group, within-subjects = objects), followed by multiple comparisons tests with statistical significance determined using the Holm-Sidak method. Microdialysis analyses were conducted using one-way ANOVA and unpaired t-student two tails. Finally, LTP analyses were measured using a one-way ANOVA and Tukey multiple comparisons test.

Fig. S1

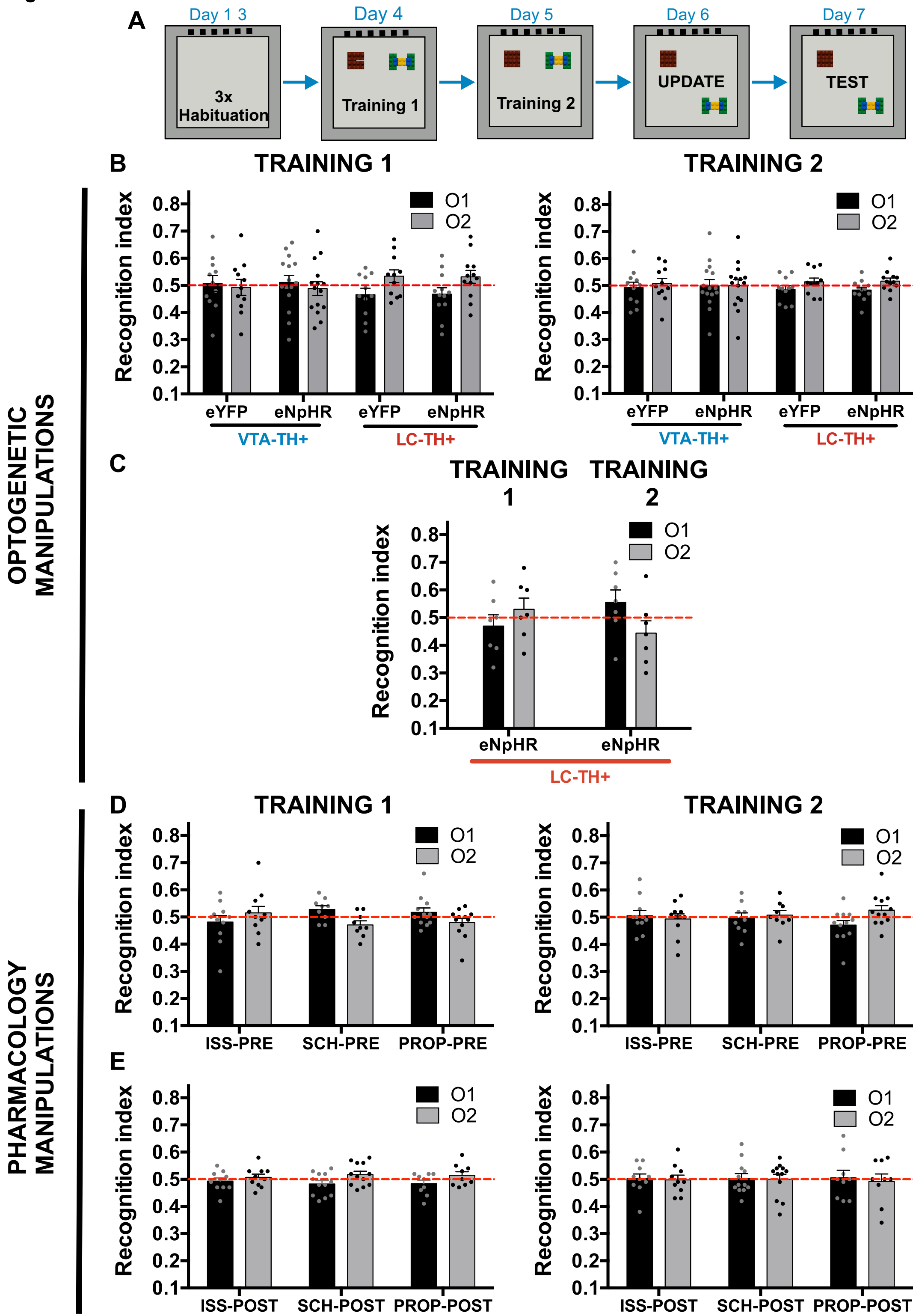

Fig. S1. *Training recognition index.* A. Protocol of object localization memory. B. Recognition index in training 1 session (group x object interaction, $F_{(3,90)} = 1.79$, $p = 0.155$) and training 2 session (group x object interaction, $F_{(3,90)} = 0.362$, $p = 0.78$) for the VTA-eYFP (n=11), VTA-eNpHR (n=15), LC-eYFP (n=11), and LC-eNpHR (n=12) groups photoinhibited in the update

session. C. Recognition index in training 1 session ($t_{(12)} = 0.999$, $p = 0.328$) and training 2 session ($t_{(12)} = 1.856$, $p = 0.146$) of LC-eNpHR (n=7) group photoinhibited after the update session. D. Recognition index in training 1 session (group x object interaction, $F_{(2,58)} = 3.13$, $p = 0.051$) and training 2 session (group x object interaction, $F_{(2,58)} = 1.881$, $p = 0.162$) in mice with ISS (n=11), SCH (n=9), and PROP (n=12) infused before update session. E. Recognition index in training 1 session (group x object interaction, $F_{(2,56)} = 0.409$, $p = 0.666$), and training 2 session (group x object interaction, $F_{(2,56)} = 0.034$, $p = 0.967$) in mice with ISS (n=10), SCH (n=12) and PROP (n=9) infused after update session. All results show the mean of recognition index ± sem.

Fig. S2

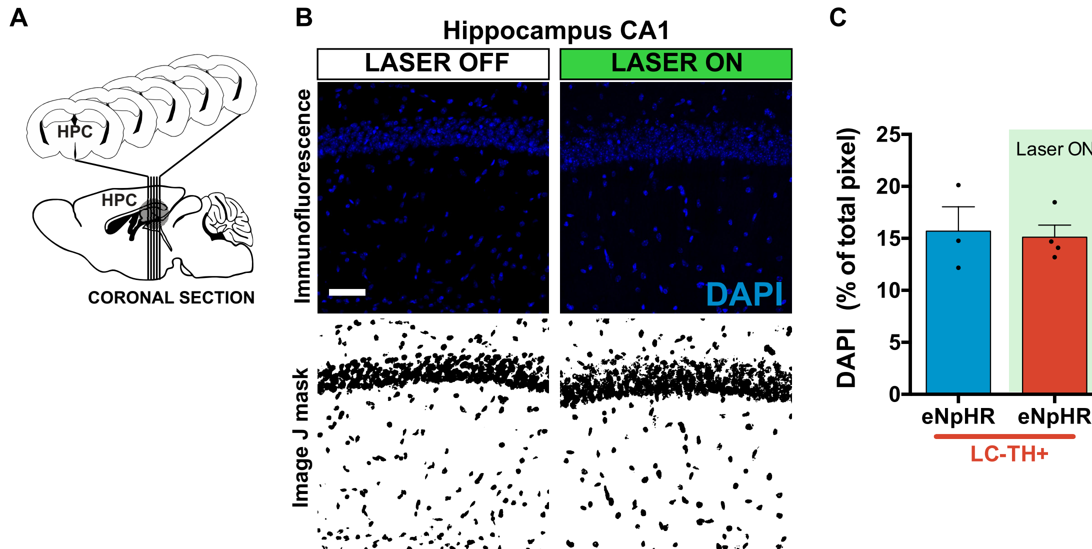

Fig. S2. *The photoinhibition does not cause changes in the hippocampal CA1 nuclei*. A. Representative coronal sections of the hippocampal CA1 analysis. B. Representative coronal immunofluorescence image of hippocampal CA1 sections with eNpHR in the LC with or without photoinhibition and Image J mask; DAPI (blue). C. Quantification of hippocampal nuclei with or without photoinhibition in the hippocampus CA1 (With n=3, without n=4; mice with six or five coronal sections). The graph showed the percentage of the total pixel area mean ± sem. Bar scale: 50 um.

Fig. S3

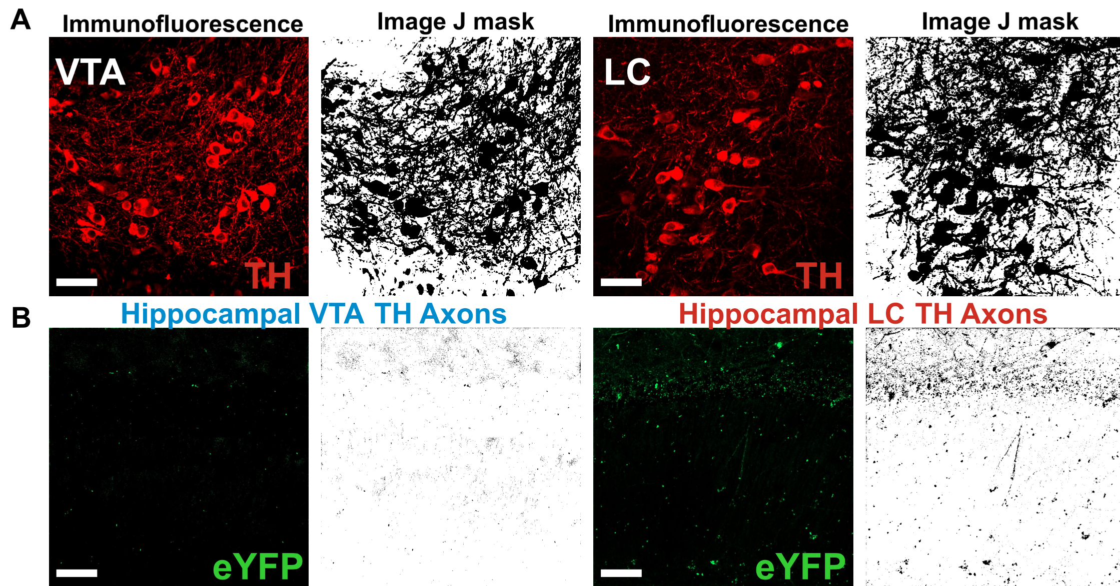

Fig. S3. *Hippocampal CA1 terminals from the LC or VTA.* A, B. Representative coronal immunofluorescence images and Image J mask of bilateral viral infection eNpHR in VTA or LC (A) and dorsal hippocampal CA1 axons from VTA or LC (B); eYFP (green) and TH (red). Bar scale: 50 um

Table S1

Table S1. *Total exploration time in optogenetic and pharmacology protocol.* Total exploration time of two objects during OLM protocol in training session 1, training session 2, update and test sessions of optogenetic and pharmacology manipulation (Green cells show optogenetic inhibition during update session). PRE shows a drug infusion before the update session, and POST shows after the update session. All results show the hundredth of a second (centiseconds, cs).

Group		Training session 1 MEAN± SD	Training session 2 MEAN± SD	UPDATE MEAN± SD	TEST MEAN± SD
Optogenetic	**VTA-eYFP**	547±187	401±255	743±343	823±358
	VTA-eNpHR	763±500	955±483	770±364	1064±359
	LC-eYFP	498±295	631±426	772±353	731±391
	LC-eNpHR	734±522	574±372	511±400	672±458
Pharmacology (PRE)	**ISS**	433±307	283±234	474±159	348±147
	SCH	645±403	285±268	309±110	318±73
	PROP	503±170	432±88	531±217	231±77
Pharmacology (POST)	**ISS**	476±253	245±202	511±314	411±155
	SCH	628±226	415±166	541±171	231±181
	PROP	276±104	435±252	625±263	392±158