Glutamatergic basolateral amygdala to anterior insular cortex circuitry maintains rewarding contextual memory

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Findings have shown that anterior insular cortex (aIC) lesions disrupt the maintenance of drug addiction, while imaging studies suggest that connections between amygdala and aIC participate in drug-seeking. However, the role of the BLA → aIC pathway in rewarding contextual memory has not been assessed. Using a cre-recombinase under the tyrosine hydroxylase (TH+) promoter mouse model to induce a real-time conditioned place preference (rtCPP), we show that photoactivation of TH+ neurons induced electrophysiological responses in VTA neurons, dopamine release and neuronal modulation in the aIC. Conversely, memory retrieval induced a strong release of glutamate, dopamine, and noradrenaline in the aIC. Only intra-aIC blockade of the glutamatergic N-methyl-D-aspartate receptor accelerated rtCPP extinction. Finally, photoinhibition of glutamatergic BLA → aIC pathway produced disinhibition of local circuits in the aIC, accelerating rtCPP extinction and impairing reinstatement. Thus, activity of the glutamatergic projection from the BLA to the aIC is critical for maintenance of rewarding contextual memory.

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The insular cortex (IC) is a region located in the temporal lobe of the brain that has been linked to the salience of stimuli, visceral control, recognition memory, and drug addiction. It has been reported that human anterior IC (aIC) lesions completely suppress smoking addiction without relapse. In addition, studies in rodents have demonstrated the participation of the aIC in the maintenance of drug addiction, although the cellular mechanisms underlying this process remain elusive.

Drugs of abuse are addictive due to the rewarding effects mediated by dopamine, a neurotransmitter released by ventral tegmental area (VTA) neurons that project to aIC and basolateral amygdala (BLA), among other areas. However, it also has been postulated that the brain’s reward circuitry is not only driven by dopaminergic neurotransmission; additional mechanisms, such as glutamatergic neurotransmission, are involved in rewarding behavior. For example, tetanic stimulation of BLA induces glutamatergic neurotransmission; additional mechanisms, such as glutamatergic neurotransmission, are involved in rewarding behavior. The glutamatergic afferents from the BLA are involved in the maintenance of contextual memories.

For example, tetanic stimulation of BLA induces glutamatergic neurotransmission; additional mechanisms, such as glutamatergic neurotransmission, are involved in rewarding behavior. The glutamatergic afferents from the BLA are involved in the maintenance of contextual memories. Several studies have suggested that consolidated memories may undergo a labile process during and after retrieval (updating), which destabilizes the original memory trace and makes it susceptible to being affected by amnestic agents, a process named reconsolidation. Interestingly, disrupting the reconsolidation of memories associated with drugs of abuse has been identified as a potential clinical target for the treatment of addiction. Accordingly, pharmacological manipulations revealed that only blockade of NMDA receptors in the aIC after the retrieval session accelerated rtCPP extinction and impaired rtCPP reinstatement. Moreover, photoinhibition of the glutamatergic BLA → aIC afferent fibers during retrieval accelerated rtCPP extinction and impaired subsequent reinstatement. Taken together, these results demonstrate that the glutamatergic BLA → aIC projection is necessary for the maintenance of rewarding contextual memories.

**Activation of VTA TH+ neurons induces rtCPP.** To test the participation of the aIC in the extinction of rewarding contextual memory, we first characterized the rtCPP induced by optogenetic stimulation of VTA TH+ neurons (Fig. 2a). We found that ChR2 mice trained in a single 5- or 20-min session developed a mild place preference (Fig. 2b). However, ChR2 mice trained for three 20-min sessions on 3 consecutive days (one session per day) developed a strong conditioned preference for the side paired with optogenetic stimulation (Fig. 2c, e). The robust place preference was maintained for five continuous extinction sessions (one per day) until the conditioned preference disappeared completely. To establish whether a reinstatement stimulation could restore the rtCPP conditioning after almost complete extinction, photoactivation was applied to ChR2 mice during a reinstatement session (ChR2/with reinstatement) in the previously conditioned side following the acquisition protocol, but in a single 5 min session (Fig. 2d). Interestingly, the magnitude of place preference displayed by ChR2/with reinstatement mice in subsequent extinction sessions was much greater than that of ChR2/without reinstatement mice. These results show that once a contextual memory is extinguished, brief photoactivation of VTA TH+ neurons is sufficient to restore the preference for the conditioned side.

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TH+ neurons in unconditioned mice (Fig. 3a), biochemical analysis revealed a strong increase of extracellular dopamine concentration (Fig. 3b). During the post-conditioning session, in the absence of the VTA TH+ neurons photoactivation (Fig. 3c), there was an increase of extracellular dopamine, noradrenaline, and glutamate concentrations (Fig. 3d). Dopamine was released during the photoactivation of the VTA TH+ neurons and during the post-conditioning session, whereas glutamate and noradrenaline were only released during the post-conditioning session, indicating that these three neurotransmitters could be involved in the retrieval of a rewarding contextual memory.

Cortical NMDA-receptor blockade accelerates rtCPP extinction. Since the extracellular concentrations of dopamine, noradrenaline, and glutamate increased in the aIC during the post-rtCPP conditioning session, we administered intra-aIC injections
of antagonists for dopamine, noradrenaline, or glutamate receptors after rtCPP retrieval session (Fig. 4a). We observed that administration of the D1-like receptor antagonist SCH23390 or the β-adrenergic receptor antagonist propranolol did not affect maintenance of rtCPP (Fig. 4b). In contrast, intra-aIC injection of AP5, an NMDA-receptor antagonist, accelerated rtCPP extinction. Interestingly, ChR2 was re-established after a reinstatement session in the ChR2/vehicle group but not in the ChR2/AP5 group (Fig. 4c). In contrast, intra-aIC injections of cyanquixaline (CNQX), a glutamate α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist, did not affect the maintenance of rtCPP (Fig. 4c). In order to know whether blockade of the NMDA receptors in the aIC after retrieval would have permanent nonspecific effects on rtCPP, we performed a reversal conditioning (Rcond 1–3) procedure. We observed that a previous blockade of NMDA receptors in the aIC did not impair the subsequent formation of new conditioning (Supplementary Fig. 2).
stimulation of the BLA induces long-term facilitation in the IC and produces a more prolonged extinction of aversive memories. In addition, microinjections of NMDA antagonists into the IC occlude changes in synaptic plasticity and long-term behavior. Altogether, these data suggest that the fibers coming from the BLA to the IC are glutamatergic and could modulate memory consolidation.

To evaluate whether silencing glutamatergic afferent fibers from the BLA to the aIC could accelerate rtCPP extinction, we performed photoinhibition of the BLA → aIC fibers during or after rtCPP retrieval in ChR2/NpHR3.0 and ChR2/eYFP mice (Fig. 5a). We photo-stimulated aIC tissue with green light (12–14 mW, 532 nm) throughout the entire post-conditioning session, regardless of the side visited by the mouse. We found that inhibition of glutamatergic afferent inputs from BLA into aIC during the post-conditioning session accelerated the extinction process (Fig. 5b). Likewise, the CPP was restored after a reinstatement session in the ChR2/eYFP but not in the ChR2/NpHR3.0 group. However, when applied after termination of a post-conditioning session, photoinhibition did not affect the maintenance or extinction of rtCPP (Fig. 5c), suggesting the existence of a critical window where BLA → aIC inputs are necessary for maintenance of the rewarding contextual memory. Finally, in a different group of naïve Chr2/NpHR3.0 mice implanted with an optrode in aIC, we found that the somata of BLA neurons expressing NpHR3.0 send projection fibers to aIC (Fig. 6a, b). We then demonstrated that 1 min of continuous photoinhibition of glutamatergic BLA → aIC fibers resulted in a robust modulation of spontaneous activity in the aIC. Figure 6c, d shows two neurons recorded in aIC: one exhibiting excitation (panel c) and the other suppression (panel d) of neuronal responses evoked by green laser photoinhibition of BLA → aIC terminals. Around 48.5% (130/268) of aIC neurons exhibited an increased firing rate, whereas 19.8% (53/268) exhibited a reduced firing rate during photoinhibition of BLA → aIC glutamatergic afferent fibers (Fig. 6e, f). Thus, photoinhibition of glutamatergic BLA → aIC afferent fibers results in a disinhibition of local cortical circuits in the aIC. Similar results were obtained in mice that only expressed NpHR3.0 in BLA in the absence of ChR2 in VTA (Supplementary Fig. 3), suggesting a lack of crosstalk between opsins. All in all, these results highlight the importance of BLA glutamatergic modulation upon aIC activity in the maintenance of rewarding contextual memory.

**Discussion**

There is extensive evidence that the mesolimbic dopaminergic system is involved in substance use disorders as well as in many forms of addiction. This system is mainly formed by dopaminergic neurons in the VTA that project to many other forebrain regions, including the aIC. Stimulation of VTA dopaminergic neurons induces behavioral and cellular hallmarks of addiction, indicating sufficiency for the induction and progression of the disease. Phasic stimulation produces action potentials of dopaminergic neurons in the VTA that are sufficient to establish a reliable rtCPP, used to measure the association of contextual reward. Optogenetic stimulation of the VTA allows for precise control of the temporal and spatial activity of dopaminergic neurons. Consequently, by using this model we were able to examine the interaction of the VTA with other relevant brain circuits participating in maintenance and extinction of rtCPP. Our findings indicate that although the firing of VTA neurons clearly covaries with each photoactivation pulse in the same structure, one conditioning session under photoactivation was not sufficient to induce a reliable rtCPP. Three conditioning
sessions of 20 min with 20 Hz photoactivation of VTA TH+ neurons were required to produce a reliable and durable rtCPP. Likewise, we found that the maintenance of rtCPP was proportional to the duration of conditioning sessions. These findings give further support to the idea that memory stability relies on repeated exposure to stimuli. In addition, our data uncovered that photoactivation of VTA TH+ neurons plays a weak neuro-modulatory role in aIC neural responses in comparison with that of VTA stimulation. Accordingly, it has been reported that activation of the D1 receptor by itself does not elicit synaptic plasticity in rat hippocampus slices, whereas high-frequency-induced LTP is facilitated or impaired by agonists or antagonist of dopamine receptors in the hippocampus and/or prefrontal cortex.

Using in vivo microdialysis, we observed that re-exposure of mice to the conditioning context during a post-conditioning session induced strong release of dopamine, noradrenaline, and glutamate. These results suggest that exposure to the conditioned context during retrieval could be the triggering factor for the release of glutamate, dopamine, and noradrenaline in the aIC. In this regard, findings obtained in experiments using other aIC-dependent behavioral tasks suggest that the release of catecholamines and glutamate during retrieval is involved in the reconsolidation and maintenance of the conditioned responses.

The phenomenon of reconsolidation posits that retrieval induces memory destabilization, creating a window in which consolidated memories are susceptible to pharmacological interference or updating with new information. In this way, after

**Fig. 4 Post-conditioning microinjections of NMDA but not AMPA-receptor antagonist accelerate rtCPP extinction.** a Diagram of virus infection, photoactivation of VTA TH+ neurons, and timeline of behavioral procedures. The preference of the compartments was determined during the pre-conditioning (Pre-cond). Mice were conditioned during three sessions 20 min of photoactivation of VTA TH+ neurons (Cond 1-3). Blue bars show photoactivation of VTA. In the post-conditioning (Post-cond), preference of the compartments was tested after optogenetic stimulation conditioning. b Microinjections of dopamine D1 (SCH23390) and β-adrenergic receptor antagonist (propranolol) into aIC after post-conditioning did not affect the maintenance (Ext 1-3) nor the reinstatement (Post-RI) of the rtCPP induced by photoactivation of 5 min (RI) of the VTA. ChR2/vehicle (n = 7), ChR2/SCH23390 (n = 7), and ChR2/Propranolol (n = 6). Two-way repeated measures ANOVA with Greenhouse-Geisser correction factor showed no group effect: F(2,17) = 0.3844, P = 0.6866, time effect: F(5,830.8976) = 59.05, P < 0.0001, and no interaction: F(30,255) = 0.5162, P = 0.9840. c Microinjections of NMDA (AP5) but not the AMPA (CNQX) receptor antagonists into the aIC after post-conditioning accelerated rtCPP extinction (Ext 1-3) and blocked the time effect: F(1,18) = 9.099, P = 0.0019, time effect: F(5,830.1049) = 103.7, P < 0.0001, and interaction: F(30,270) = 2.216, P = 0.0005. Post hoc Tukey test ChR2/Vehicle vs. ChR2/AP5 for Ext 1 P = 0.0025, Ext 2 P = 0.0028, Ext 3 P = 0.0234, Ext 1-RI P = 0.0103. All data are shown as mean ± SEM. The arrow indicates the time of administration of the drugs. Dashed horizontal lines indicate no preference. *P < 0.05, **P < 0.01.
Fig. 5 Photoinhibition of inputs from the BLA to the aIC accelerate rtCPP extinction. a Diagram of virus injection into VTA TH+ neurons, photoinhibition of BLA neurons, and timeline of behavioral procedures. The preference of the compartments was determined during the pre-conditioning (Pre-cond). Mice were conditioned during three sessions 20 min of photoactivation of VTA TH+ neurons (Cond 1-3). Blue bars indicate photoactivation of VTA; green bar indicates photoinhibition of aIC. b Photoinhibition of the aIC during the post-conditioning (Post-cond) accelerated extinction in the ChR2/NpHR3.0 (n = 9) but not in the ChR2/eYFP (n = 7) group. Green bar shows photoinhibition of the aIC. The green laser was constantly pulsed at 12-14 mW and 532 nm. Two-way repeated measures ANOVA with Greenhouse–Geisser correction factor showed main group effect: F1,14 = 7.266, P = 0.0174, time effect: F4,704,65.85 = 62.50, P < 0.0001, and interaction: F15,210 = 4.406, P < 0.0001. Post hoc Bonferroni ChR2/eYFP vs. ChR2/NpHR3.0 for Ext 1 P = 0.0042, Ext 2 P = 0.0068, post-RI P = 0.0483, Ext 2-RI P = 0.0082, Ext 3-RI P = 0.0206, Ext 4-RI P = 0.0013. c Photoinhibition of the aIC after post-cond did not affect the maintenance of the rtCPP in any of the two groups, NpHR3.0 (n = 7) and eYFP (n = 7). Two-way repeated measures ANOVA with Greenhouse–Geisser correction factor showed no group effect: F1,12 = 1.178, P = 0.229, time effect: F5,815,69.70 = 29.01, P < 0.0001, and no interaction: F15,180 = 0.6331, P = 0.8450. All data are shown as mean ± SEM. The arrow indicates the time of inhibition of BLA → aIC circuitry. Dashed horizontal lines indicate no preference. *P < 0.05, **P < 0.01.

retrieval, new information is compared with the already stored memories, which can thus be destabilized. Subsequently, both the destabilized memory and the incoming information are re-stabilized or reconsolidated into an updated long-term memory. In the rtCPP model, when mice are re-exposed to the learning environment a labile conditioned memory could be affected by amnesic drugs such as NMDA antagonists, impairing previous memory, and accelerating rtCPP extinction. Such results have been reported with fear conditioning, object recognition memory, and traditional CPP. We found that NMDA receptors are critical for maintaining rtCPP, whereas D1, β-adrenergic, and AMPA receptors do not appear to participate in the maintenance of rtCPP memory. Blockade of NMDA receptors in the aIC may destabilize the original memory trace, thereby accelerating extinction and impairing reinstatement to allow the acquisition of new reinforcement learning. This possibility is supported by evidence showing that reconsolidation mechanisms in recognition memory require the activation of NMDA but not AMPA receptors. Our results further demonstrate that intra-aIC administration of NMDA-receptor antagonists produced a reliable and long-lasting impairment of rtCPP maintenance. Therefore, it is possible that the aIC could be an important hub that links contextual and visceral information related to the maintenance of memories associated with addictive drugs.

Findings of several experiments suggest important communication between the amygdala and the IC during appetitive and aversive memory formation. For example, a reliable LTP in the aIC was induced by high-frequency stimulation in the BLA, which was blocked by intracortical administration of an NMDA antagonist. These results suggest that cortical plasticity induced by amygdala stimulation could be regulated by glutamate through activation of NMDA receptors in the aIC. Therefore, we
Fig. 6 Continuous photoinhibition of glutamatergic → aIC fibers provoke disinhibition of local cortical circuits in the aIC. a Diagram of virus infection and diffusion throughout BLA anteroposterior axis. b Representative images of double immunofluorescence for eYFP (green) and 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI; blue) in basolateral amygdala (BLA) somas (left panels) and anterior insular cortex (aIC) axons (right panels). White scalebar represents 100 µm. AICd agranular insular cortex dorsal, AICv agranular insular cortex ventral. c Raster plot and corresponding PSTH (below) of a representative excited neuron recorded in aIC. Responses were aligned (time = 0) to onset of photoinhibition for 1 min (shown by green rectangle). Black ticks indicate action potentials. Top, scheme of the experimental protocol. d Representative aIC neuron exhibiting suppression during photoinhibition. e Z-score normalized color-coded PSTH of neurons with either increased (Inc) or decreased (Dec) evoked responses recorded in the aIC while the glutamatergic BLA → aIC fibers were opto-inhibited. Red color indicates higher firing rates, whereas blue color inhibitory responses. Lines depict the start and the end of photoinhibition of green laser. f Mean Z-scores of neuronal populations categorized by significant increase (inc), decrease (dec), or no effect during photoinhibition.

In summary, by employing optogenetic and behavioral approaches, we report that inhibition of the BLA → aIC pathway accelerates the extinction and impairs the re-establishment of a rewarding contextual memory. We observed a similar behavioral pattern by the pharmacological blockade of NMDA receptors in the aIC. Our data strongly suggest that the BLA → IC pathway is centrally involved in the maintenance of rewarding contextual memory through NMDAR-dependent glutamatergic signaling.

**Methods**

**Animals.** TH-Cre mice (TH, FlI12 line) express Cre-recombinase protein under control of the endogenous TH promoter. Breeder mice were kindly donated by Dr Rui M. Costa from the Champalimaud (Center for the Unknown) and crossed onto C57BL/6J mice for at least six generations. Male and female experimental TH-Cre mice were 3 to 4 months old (23–35 g body weight) at the initiation of experiments. No differences were found between male and female TH-Cre mice in any of the experiments performed. Mice were individually housed with food and water available ad libitum in a light/dark cycle room (12:12 h) with temperature of 22 ± 2 °C and relative humidity of 50 ± 5%. The behavioral tests were carried out during the light phase of the cycle. All procedures were approved by the Instituto de Fisiologia Celular (EFR125-18) Institutional Animal Care and Use Committee and complied with the guidelines established in the Official Mexican Standard (NOM-062-ZOO-1999).

**Genotyping.** We used the HotSHOT method for DNA extraction. The method consists of the lysis of 1 mm of a tail snap in an alkaline reagent (NaOH 25 mM, diodium EDTA 0.2 mM) under heat conditions (95 °C, 1 h) and further neutralization with a suitable buffer (Tris-HCl 1 M at pH 7.5). After centrifugation at 2500 rpm for 2 min, the supernatant containing the DNA was recovered. This DNA was used for amplification through polymerase chain reaction (PCR) with the Qiagen kit. The primers used for PCR were Cre Reverse 5′-AGCCTGTTTTGCACGTTCACC and Cre Forward 5′-GAACCTGAA. The Cre-inducible adeno-associated virus (AAV) was obtained from the University of North Carolina (UNC) Gene Therapy Center Vector Core. The viral concentration was 5.2 × 10^11 virus molecules ml^-1 for rAAV5/Ef1a-DIO-hChR2(H134R)-eYFP (ChR2), 6.0 × 10^11 virus molecules ml^-1 for rAAV5/Ef1a-DIO-eYFP (eYFP), 5.2 × 10^11 virus molecules ml^-1 for rAAV5-CamKIIa-eNpHR3.0 (NpHR3.0), and 5.1 × 10^11 virus molecules ml^-1 for rAAV5-CamKIIa-eYFP (eYFP). Viruses were subdivided into aliquots stored at ~80 °C until use.

**Stereotaxic surgery.** Mice were anesthetized with isoflurane (induction 3%, maintenance 1–1.5%, VIP 3000 Matrix). The microinjection needles (29-G) were connected to a 10 µl Hamilton syringe and filled with AAV. For all experiments, the mice were bilaterally injected with AAV (0.5 µl) at a rate of 0.1 µl min^-1 with additional 3 min for a complete diffusion and implanted with 200 µm core optic fibers through 1.25-mm-wide zirconia ferrules in each hemisphere. The ChR2 or eYFP vectors were injected into the VTA (from Bregma (mm): −3.08 AP, ±0.60 ML, and −4.80 DV). The NpHR3.0 or eYFP vectors were injected into the BLA (from Bregma (mm): −2.65 AP, ±3.40 ML, and −5.30 DV). The optic fibers were implanted above the VTA (from Bregma (mm): −3.08 AP, ±1.20 ML, and −4.30 DV at 10° angle) or the aIC (1.70 AP, ±2.60 ML, and −2.75 DV). For microdialysis experiments, the mice were implanted unilaterally with a guide cannula (CMA/7: 2500 rpm by 2 min, the supernatant containing the DNA was recovered. This DNA was used for amplification through polymerase chain reaction (PCR) with the Qiagen kit. The primers used for PCR were Cre Reverse 5′-AGCCTGTTTTGCACGTTCACC and Cre Forward 5′-GAACCTGAA. The Cre-inducible adeno-associated virus (AAV) was obtained from the University of North Carolina (UNC) Gene Therapy Center Vector Core. The viral concentration was 5.2 × 10^11 virus molecules ml^-1 for rAAV5/Ef1a-DIO-hChR2(H134R)-eYFP (ChR2), 6.0 × 10^11 virus molecules ml^-1 for rAAV5/Ef1a-DIO-eYFP (eYFP), 5.2 × 10^11 virus molecules ml^-1 for rAAV5-CamKIIa-eNpHR3.0 (NpHR3.0), and 5.1 × 10^11 virus molecules ml^-1 for rAAV5-CamKIIa-eYFP (eYFP). Viruses were subdivided into aliquots stored at ~80 °C until use.

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General procedures. The behavioral experiments began 3–4 weeks after surgery for the groups injected in the VTA to allow sufficient expression of opsin. They received optogenetic stimulation in the VTA or aIC through optical tethers, which consisted of a 26 gauge, solid-state blue (473 nm) and green (532 nm) laser (OEM Laser Systems) coupled to 62.5 μm core, 0.22 NA standard multimode hard-cladding optical fiber (Thorlabs) that passed through a single-channel optical rotary joint (Doric Lenses) prior to being split 50:50 with a fused optical coupler. The intensity of light output was 12–15 mW per split fiber for all experiments. The mice were connected to the laser just before starting each behavioral or microdialysis session. For pharmacological studies, DL-2-amino-5-phosphonopentanoic acid (10 mg ml⁻¹, DL-AP5, #P0105, Tocris Bioscience), an N-methyl-D-aspartate (NMDA) receptor antagonist; cyanuric acid (1 mg ml⁻¹, CNQX, disodium salt hydrate, #C239, Sigma-Aldrich), an α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) antagonist; lidocaine (2 mg ml⁻¹, #S723390, hydrochloride, #D054, Sigma-Aldrich), a dopamine D1 receptor antagonist and DL-Propranolol hydrochloride (5 mg ml⁻¹, #P0884, Sigma-Aldrich), a β-adrenergic receptor antagonist, were dissolved in 0.9% saline solution. All drugs were injected into aIC immediately after the post-conditioning session at a rate of 0.25 μl min⁻¹ for 1 min laser on and 1 min off for 30 min (constant pulsation at 12 Hz). The samples were injected hydro-dynamically at 0.5 psi for 5 s; then the capillary they move through a window where an argon-ion laser excites them at 488 nm. Then fluorescence was filtered by a band-pass interference filter at 590 nm and detected by a photomultiplier tube. The signal was translated into peaks that form an electropherogram where they can be analyzed by comparing the area under the curve (AUC) of the unknown sample with the AUC of the maximum peak as determined from standard. Data were analyzed using the Karat System Gold (Beckman Coulter, version 5.0. All results were converted into a percentage of baseline release (percentage baseline = neurotransmitter concentration × 100/mean of the three first samples).

Immunofluorescence. Mice were sacrificed with sodium pentobarbital (75 mg/kg), perfused with 0.9% saline solution and fixed with 4% paraformaldehyde solution. Brain was quickly removed and stored in 4% paraformaldehyde solution. Leica CM520 cryostat was used to obtain 40 μm coronal slices. Free-floating sections were mounted in Dako fluorescence mounting medium with 4′,6-diamidino-2-phenilindol. Immunofluorescence was observed using a ZEISS LSM 800 confocal microscope.

Optrode recordings during photoactivation of VTA neurons. TH-Cre mice (ChR2, n = 11) were injected with ChR2 bilaterally into the VTA (from Bregma (mm): −3.0 AP, ±0.60 ML and −4.80 DV) and 3 weeks of recovery were allowed for ChR2 expression. After this time, eight mice were implanted bilaterally with optic fibers at VTA (from Bregma (mm): −3.0 AP, ±1.20 ML, and −4.30 DV, 10° angle) as described above, but in this case, an array of 16 tungsten wires was implanted unilaterally in the aIC (from Dura (mm): −1.4 AP, ±3.30 ML, and −3.20 DV). Another group of mice (n = 3) were implanted with a homemade optrode (16 tungsten wires, 35 μm each), surrounding the optic fiber in the VTA (from Dura (mm): −3.0 AP, ±0.60 ML, and −4.0 DV). Mice were allowed 1 additional week to recover. Mice were recorded in a scanner laser frequency task, where they received a random frequency of stimulation during 1 s on, followed by 2 s off, and the next frequency was randomly chosen. The frequencies of stimulation were: control (no stimulation), 4, 7, 20, 33, 50 Hz (5 ms pulse width), and a 1 s continuous pulse. The task was carried out for 20 min. Mice were recorded in maximum of nine consecutive sessions.

Optrode recordings during photoinhibition of BLA → aIC fibers. One group of TH-Cre mice (NpHR3.0/ChR2, n = 3) were injected with ChR2 bilaterally into the VTA (from Bregma (mm): −3.08 AP, ±0.60 ML, and −4.80 DV), NpHR3.0 bilaterally into the BLA (from Bregma (mm): −2.05 AP, ±3.40 ML, and −5.30 DV), and 3 weeks of recovery were allowed for ChR2 and NpHR expression. After that, an optrode was implanted unilaterally in the aIC (from Dura (mm): ±1.4 AP, ±3.30 ML, and −3.20 DV). Another group of TH-Cre mice (NpHR3.0, n = 5) were injected only with NpHR3.0 bilaterally into the BLA (from Bregma (mm): −2.0 AP, ±3.40 ML, and −5.30 DV), and an optrode was implanted in aIC (from Dura (mm): ±1.4 AP, ±3.30 ML, and −3.20 DV) after 3 weeks. After surgery, mice were allowed 1 additional week for recovery. Then both groups of mice were recorded and photoinhibited into aIC during an open-loop task that consisted of blocks of 1 min laser on and 1 min off for 30 min (constant pulsed at 12–14 mW and 532 nm), in seven consecutive sessions.

Extracellular recordings. Neural activity was recorded using a Multichannel Acquisition Processor system (Plexon, Dallas, TX) interfaced with a Med Associates conditioning side of the rtCPP to record behavioral events simultaneously. Capacitive voltage signals were first amplified ×1 by an analog headstage (Plexon HST/1625-GEN2-18P-2GP-C1), then amplified (×1000) and sampled at 40 kHz. Raw signals were pass-band filtered from 154 to 8.8 kHz and digitalized at 12 bits resolution. Only single neurons with action potentials with a signal-to-noise ratio of 3:1 were analyzed. The action potentials were isolated on-line using voltage-time threshold windows and three principal components contour templates algorithm. A cluster of waveforms was assigned to a single unit if two criteria were met: inter-spike intervals were larger than the refractory period set to 1 ms, and if a visible ellipsoid cloud composed of the 3-D projections of the first three principal component analysis of spike waveform shapes was formed. Spikes were sorted using Sorter software (Plexon, Dallas, TX). Spike train time stamps from offline-sorted waveforms were analyzed. All electrophysiological data were analyzed using MATLAB (The MathWorks Inc., Natick, MA).
**Statistics and reproducibility.** Behavioral and microdialysis data were analyzed in GraphPad Prism 8. When the data were normally distributed according to the Kolmogorov–Smirnov test, a repeated measure two-way ANOVA was used with the Geisser–Greenhouse correction for sphericity. Bonferroni and Tukey’s multiple comparisons test was performed when appropriate. Non-normally distributed data were analyzed using Mann–Whitney test. All data are shown as mean ± SEM. α was always set p < 0.05. All statistical tests used were two-tailed tests. The electrophysiological analysis was performed using Matlab 2015a. In the case of electrophysiological recordings, the firing rate was compared using a nonparametric Kruskal–Wallis test followed by a Tukey–Kramer post hoc. Neurons whose firing rates were significantly different (p < 0.05) from the control trial during the laser “on” period at any laser frequency were categorized as modulated. The difference between firing rates during laser stimulation vs. control trials determined the sign of the modulation (i.e., increased or decreased).

For behavioral experiments, sample size was the minimum necessary to get an effect size threshold of 0.5 using the Cohen’s d test dividing the difference between the means and the pooled standard deviation. Due to our previous experience in microdialysis studies, we used the minimal number of mice to get a statistical significance using nonparametric analysis. For electrophysiological recordings our sample size was based on similar previous studies and all groups were replicated independently. Data analysis was performed by blind observers, that is, mice were identified only after getting the behavioral, electrophysiological or microdialysis parameters.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The datasets and codes generated during and/or analyzed during the current study are available in the Figshare repository, https://doi.org/10.6084/m9.figshare.11418195.v1.

**Code availability**

Code was written in Matlab 2015a and it is available upon request from the corresponding author.

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Author contributions
E.G.L., I.B., F.T., R.G., A.B., R.A.McD. and F.B-R. designed the research. E.G.L., I.B., and P.M.C. performed in vivo microdialysis, behavioral, and optogenetic experiments. E.G.L. and I.I-L. performed in vivo optrode electrophysiological experiments. E.G.L. and I.B. performed imaging analysis. E.G-L., R.G., A.B., R.A.McD. and F.B-R. wrote the paper. E.G-L. created the mice, boxes, and brain images. All experiments were done in Mexico City labs. All mice and boxes elements were created by E.G.-L., brain images were vectorized using a public domain sagittal brain image as template, and coronal brain pictures were modified from Allen’s reference atlas of the mouse brain.

Competing interests
The authors declare no competing interests.

Additional information
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