

Oxytocin Activity in the Paraventricular Nucleus Facilitates Social Recognition Memory via Lateral Septum Neurons in an Ovariectomized Mouse

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Abstract

Oxytocin (OXT) is a key modulator of social recognition memory (SRM), yet the responsiveness of OXT neurons to SRM processes in ovariectomized (OVX) mice remains unclear. In this study, we first observed that SRM was significantly impaired in OVX mice 4 to 8 weeks post-surgery. Immunostaining analyses revealed that OXT neurons in the hypothalamic paraventricular nucleus (PVN) were activated after OVX. Notably, chemogenetic inhibition of PVN^{OXT} neurons substantially alleviated SRM impairment in OVX mice. Quantitative real-time polymerase chain reaction (qRT-PCR) assays showed a marked upregulation of oxytocin receptor (OXTR) expression in the lateral septum (LS), along with increased neuronal activity in this region. To explore the underlying mechanism, we investigated the functional role of PVN^{OXT} neuron projections to the LS. Chemogenetic inhibition of these PVN^{OXT}-LS projections improved SRM deficits in OVX mice. Inhibition of LS neurons receiving input from PVN^{OXT} neurons significantly enhanced SRM in OVX mice. Finally, treatment with an OXTR antagonist in the LS of OVX mice exerted a similar ameliorative effect on SRM. Collectively, these findings indicate that PVN^{OXT} neurons regulate SRM in OVX mice via projections to the LS, highlighting a critical role of PVN^{OXT} neurons in estrogen withdrawal-induced SRM impairment.

1. Introduction

Social recognition memory (SRM) is essential for gregarious or group-living animals to identify and distinguish conspecifics¹. As a core component of episodic memory, SRM enables social animals to exhibit contextually appropriate social behaviors. For example, when a male mouse encounters a more aggressive conspecific that previously defeated it, the memory of this defeat prompts avoidance behavior². Thus, retaining information about distinct conspecifics is a vital foundation for SRM.

Several studies have demonstrated that estrogen influences social recognition processes^{3,4}. Female mice in the proestrous phase, characterized by elevated circulating estrogen levels, perform better in the habituation-dishabituation paradigm used to assess social recognition⁵⁻⁷. Previous studies have reported that estrogen could regulate oxytocin (OXT) expression^{8,9}, which is a neuroendocrine nonapeptide mainly secreted by the paraventricular nucleus (PVN) and supraoptic nucleus (SON). However, its functions as a neuromodulator to regulate SRM in estrogen withdrawal condition was unknown.

Across various mammalian species, brain regions associated with social behavior have a higher density of OXT-immunoreactive nerve fibers in females than in males¹⁰⁻¹⁴, and OXT activity in the PVN is critical for SRM in mice¹⁵. Sexual dimorphisms in the central OXT system are thought to be linked to sex-specific estrogen levels^{6,8,9,16,17}. Despite extensive research on oxytocin and its general role in social behaviors, our understanding of the precise role of PVN^{OXT} neurons in SRM remains incomplete. Existing studies have only implied a critical role of PVN^{OXT} neuron activity in social memory^{15,18,19}. However, the regulatory mechanisms of PVN^{OXT} neurons in SRM in the OVX mouse model remain unclear.

In the current study, we observed increased activity of PVN^{OXT} neurons in OVX mice. Chemogenetic suppression of these neurons improved SRM in OVX mice. Further investigations revealed upregulated mRNA expression and enhanced activity of OXTR in the LS. Notably, chemogenetic inhibition of PVN^{OXT} neurons projecting to the LS enhanced SRM, while inhibition of LS neurons receiving inputs from PVN^{OXT} neurons impaired SRM. Finally, using a highly specific OXTR antagonist, we confirmed that OXTR signaling in the LS is critical for SRM. Taken together, these results indicate that the PVN^{OXT}-LS neural circuit modulates social memory in OVX mice.

2. Results

2.1. Estrogen withdrawal impairs SRM in OVX mice

To investigate estrogen's role in SRM, we established an ovarian estrogen withdrawal model in female mice. SRM-related behaviors were evaluated at 4, 6, and 8 weeks post-ovariectomy using the three-chamber test. OVX mice showed a significant alteration in the social discrimination index from 4 to 8 weeks after estrogen depletion (Fig. 1B), whereas the social preference index remained unchanged (Fig. 1A). Based on these observations, mice at 6 weeks post-ovariectomy were selected as the model for subsequent experiments (Fig. 1C-E). Additionally, estrogen withdrawal induced depressive-like phenotypes in OVX mice after 6 weeks post-surgery (Fig. S1). Collectively, these results demonstrate that estrogen withdrawal elicits depressive-like behaviors and impairs SRM in female mice.

2.2. PVN^{OXT} neurons respond to estrogen in OVX mice

To examine the direct role of PVN^{OXT} neurons in OVX-induced SRM impairment, we explore the activity of PVN^{OXT} neurons. Immunostaining of OXT neurons in conjunction with c-Fos revealed that the activation of PVN^{OXT} neurons and their number increased in OVX mice compared with the sham group (Fig. 2A-C). Meanwhile, the mRNA level of OXT was enhanced in OVX mice compared with the sham group (Fig. 2D). Additionally, we found that the positive OXT neurons were increased by ICH staining (Fig. 2E-F). Overall, these results demonstrated that the PVN^{OXT} neurons were activated in OVX mice.

2.3. Inhibition of PVN^{OXT} neurons improves estrogen withdrawal-induced social memory impairment in OVX mice

Given that estrogen withdrawal enhanced the activity of PVN^{OXT} neurons in OVX mice, we employed chemogenetic approaches to inhibit these neurons. Specifically, AAV-EF1 α -DIO-hM4Di-WPRE was stereotactically injected into the PVN of OXT-IRES-Cre mice, enabling selective inhibition of OXT neurons (Fig. 3A). Four weeks post-viral injection, administration of DCZ effectively suppressed PVN^{OXT} neuron activity (Fig. 3B-C). In the three-chamber test, inhibition of PVN^{OXT} neurons significantly increased the

investigation time compared with the saline group (Fig. 3D, 3F), while exerting no significant effect on the interaction time (Fig. 3E). Notably, inhibition of PVN^{OXT} neurons did not alter depression-like behaviors in OVX mice (Fig. S2). Collectively, these findings demonstrate that inhibition of PVN^{OXT} neurons ameliorates estrogen withdrawal-induced SRM impairment without modulating depression-like behaviors in OVX mice.

2.4. OXTR neurons respond to estrogen in OVX mice

PVN^{OXT} neurons project to brain regions involved in social behavior regulation, including the medial prefrontal cortex (mPFC), LS, hippocampus, and amygdala²⁰. To investigate downstream signaling mechanisms of PVN^{OXT} neurons, qRT-PCR was performed to assess OXTR expression in these target regions. Results showed significantly increased OXTR mRNA expression in the LS of OVX mice compared with the sham group (Fig. 4A-B). Furthermore, OXTR neurons were activated in the LS in OVX mice (Fig. 4C-E). These findings demonstrate that OXTR in the LS is functionally upregulated and may contribute to PVN^{OXT} neuron-mediated regulation of social-related neural activity in OVX mice

2.5. The PVN^{OXT}-LS circuit regulates SRM in OVX mice

To confirm the conjunction between PVN^{OXT} neurons and the LS, we injected AAV-DIO-EGFP into the PVN (Fig. 5A). Four weeks later, EGFP was successfully expressed in PVN^{OXT} neurons, and LS obtained the projection from PVN^{OXT} neurons (Fig. 5B). Given the increased OXTR level in the LS, we hypothesized that LS^{OXTR} neurons contribute to SRM in OVX mice. To investigate the role of the PVN^{OXT}-LS circuit, AAV2/1-EF1 α -DIO-FLP was injected into the PVN of OXT-IRES-Cre mice, and AAV2/9-fDIO-hM4Di-mCherry was administered into the LS (Fig. 5C). Four weeks later, chemogenetic inhibition of LS neurons receiving input from PVN^{OXT} neurons was achieved following DCZ injection (Fig. 5D). Inhibition of these LS neurons increased time spent with novel mice, and without affecting interaction time with the mouse in OVX mice (Fig. 5E-G). These results demonstrate that inhibition of LS neurons receive projection from PVN^{OXT} neurons that regulate SRM in OVX mice.

To further explore the role of PVN^{OXT} neurons projecting to the LS, we employed flippase recombinase (FLP)-dependent viral vectors for targeted modulation (Fig. 6A). Specifically, AAV2/9-fDIO-hM4Di was bilaterally administered into the PVN, and Retro-DIO-FLP was injected into the LS of OXT-IRES-Cre mice. Four weeks later, DCZ injection inhibited PVN^{OXT} neurons (Fig. 6B-C). Chemogenetic inhibition of PVN^{OXT} neurons projecting to the LS increased time spent interacting with novel mice without affecting social preference (Fig. 6D-F). These results demonstrate that the PVN^{OXT}-LS circuit plays a crucial role in mediating SRM in OVX mice.

2.6. Injection of an OXTR antagonist in the LS improves social memory in OVX mice

To confirm whether OXT downstream signaling in the LS is necessary for social memory, we infused a selective OXTR antagonist ([d(CH₂)⁵1, Tyr(Me)², Thr⁴, Orn⁸, des-Gly-NH²⁹], OXTA: 0.3 µl, 75 ng total) into the LS (Fig. 7A)¹⁵. OVX mice injected with saline showed no preference for novel over familiar mice, whereas those infused with the OXTR antagonist preferred novel mice (Fig. 7B-D). These results suggest that OXT signaling in the LS is critical for regulating SRM.

2.7. Input Sources to PVN^{OXT} neurons

To identify input sources to PVN^{OXT} neurons, we stereotactically injected rAAV-EF1α-DIO-H2BEGFP-T2A-TVA and rAAV-EF1α-DIO-ΔRVG into the unilateral PVN of OXT-IRES-Cre mice (Fig. 8A). After 21 d, RV-CVSENVN-N2C(ΔG)-tdTomato was injected into the same site (Fig. 8A). Eight days later, immunohistochemical analysis confirmed successful trans-synaptic labeling (Fig. 8B). We identified direct projections to PVN^{OXT} neurons from several ipsilateral brain regions, including the medial preoptic area (MPOA), bed nucleus of the stria terminalis (BNST), lateral hypothalamus (LH), dorsomedial hypothalamus (DMH), hippocampus, and zona incerta (ZI) (Fig. 8C). These regions express estrogen receptors and are previously implicated in social behavior regulation. These findings suggest that PVN^{OXT} neurons may integrate aversive event information from multiple sources.

3. Discussion

Our study investigated the regulatory mechanism of SRM in female mice following ovarian estrogen withdrawal, providing novel insights into the neural circuits and molecular pathways underlying estrogen-dependent social cognition. This is the first study to confirm that the PVN^{OXT}-LS circuit serves as a key neural pathway mediating estrogen withdrawal-related changes in SRM, while also revealing the core role of OXT and OXTR within this circuit. These findings provide novel experimental evidence for understanding the mechanism underlying the synergistic regulation of SRM by estrogen and neural circuits.

SRM is a complex behavioral process that requires the coordinated involvement of multiple brain regions to store and retrieve social and spatial information²¹. Brain regions including the mPFC, LS, and hippocampus are implicated in both social recognition and stress responses, reflecting the interconnected nature of these neural networks^{19,22–26}. Our research demonstrated that estrogen withdrawal induced the expression of c-Fos (a marker of neuronal activation) in PVN^{OXT} neurons, and inhibition of these neurons alleviates social memory impairment. These observations suggest that estrogen withdrawal-induced upregulation of PVN^{OXT} neuron activity may disrupt the normal function of social recognition networks. Importantly, administration of an OXTR antagonist in the LS mimicked the beneficial effects of PVN^{OXT} neuron inhibition, underscoring the critical role of the PVN^{OXT}-LS axis in this experimental paradigm. Collectively, these data imply that activation of PVN^{OXT} neurons under estrogen withdrawal conditions may remodel brain-wide neural networks involved in social recognition, leading to impaired social memory.

The neuropeptide OXT has long been considered a potential therapeutic agent for alleviating social recognition deficits, based on its well-documented role in promoting social behavior in various contexts^{15,19,27}. Under normal physiological conditions, inhibition of PVN^{OXT} has no effect on social discrimination. However, in restraint-stressed mice, suppressing PVN^{OXT} can ameliorate social discrimination¹⁹. Our research uncovers a contrasting phenomenon: heightened activity of PVN^{OXT} neurons under estrogen withdrawal conditions exacerbates social recognition impairment, and this impairment can be reversed by inhibiting PVN^{OXT} neurons or blocking OXTR signaling in the LS. These results challenge the conventional view of OXT as a universal promoter of SRM and suggest that its role may be context-dependent¹⁵. Our study provides a potential explanation for these inconsistent results, highlighting that the functional role of OXT may vary depending on the underlying neuroendocrine context (e.g., estrogen status) and the specific neural circuits involved.

Furthermore, this study identified extensive inputs to PVN^{OXT} neurons from multiple brain regions, including the MPOA, BNST, LH, DMH, hippocampus, and ZI. All these brain regions express estrogen receptors and are known to regulate social behavior^{28–30}, suggesting that PVN^{OXT} neurons act as an "integrative center" for estrogen-sensitive social information. For example, the MPOA is involved in processing gender-related social cues, while the hippocampus is critical for memory encoding and retrieval^{28,31,32}. By integrating information from these diverse regions, PVN^{OXT} neurons can modulate social memory through their projections to the LS, providing a crucial anatomical basis for understanding how estrogens regulate SRM via complex neural networks. This circuit structure—characterized by a variety of inputs and specific outputs—highlights the sophistication of estrogen-dependent SRM regulation and opens new avenues for exploring the interplay between hormonal and neural systems in social behavior.

In conclusion, our study reveals that the PVN^{OXT}-LS circuit mediates estrogen withdrawal-induced SRM impairment in female mice, with OXT and OXTR serving as core molecular mediators. Our findings challenge the conventional view of OXT as a universal enhancer of SRM and highlight the context-dependent nature of its function. Additionally, we demonstrate that PVN^{OXT} neurons integrate social information from multiple estrogen-sensitive brain regions, providing a comprehensive framework for understanding estrogen-dependent SRM regulation. These results not only advance basic neuroscience research but also identify potential therapeutic targets for SRM impairments associated with estrogen withdrawal.

4. Materials and Methods

4.1 Animals

Female C57BL/6J mice were purchased from GemPharmatech Co., Ltd., and OXT-IRES-Cre mice were obtained from Shanghai Model Organisms Center Inc. All animals were maintained in a specific pathogen-free (SPF) barrier facility. OXT-IRES-Cre mice were housed in a breeding configuration (1 male,

2 females per cage), while other experimental mice were group-housed (5 mice per cage). All animals were kept under a 12/12-hour light-dark cycle (lights on: 9:00–21:00) with ad libitum access to food and water. Following ovariectomy, mice were singly housed for one week to facilitate postoperative recovery. All animal experiments were conducted in compliance with the 3R principles (Replacement, Reduction, Refinement) and during the light phase (9:00–17:00). The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Xuzhou Medical University.

4.2 Establishment of OVX Model

Female mice were anesthetized with isoflurane and subjected to bilateral abdominal incisions for the exposure and removal of the ovaries (OVX). To validate the model, the mice were divided into two groups: the Sham group and the OVX group.

4.3 Stereotactic surgery

Stereotactic coordinates were as follows: PVN: anteroposterior (AP) + 0.9 mm, mediolateral (ML) $\pm$ 0.2 mm, dorsoventral (DV) – 4.75 mm; LS: AP 0.5 mm, ML $\pm$ 0.25 mm, DV 2.5 mm.

4.4 Viral Injection

To achieve chemogenetic modulation of PVN^{OXT} neurons and the neurons in the LS region that receive their projections, the following recombinant adeno-associated viruses (rAAVs) were injected into the PVN: rAAV-EF1 α DIO-hM4D(Gi)-mCherry-WPRE-hGH-polyA (6.45×10^{12} vg/ml, PVN injection volume 200 nl).

For chemogenetic modulation of the PVN-to-LS projection pathway, AAV2/R-EF1 α -DIO-FLP-WPRE-hGH pA (5.50×10^{12} vg/ml, injection volume 100 nl) was injected into the LS of OXT-IRES-Cre mice, and rAAV-hSyn-fDIO-hM4D(Gi)-mCherry-WPRE-hGH-polyA (5.27×10^{12} vg/ml, PVN injection volume 200 nl) was injected into the PVN. Additionally, AAV2/1-EF1 α -DIO-FLP-WPRE-hGH pA (5.5×10^{12} vg/ml, injection volume 200 nl) was injected into the PVN, and rAAV-hSyn-fDIO-hM4D(Gi)-mCherry-WPRE-hGH-polyA (5.27×10^{12} vg/ml, LS injection volume 150 nl) was injected into the LS. After 4 weeks of viral injection, chemogenetic modulation was performed as follows: mice carrying the hM4D(Gi) receptor were injected intraperitoneally with DCZ (dose 100 μ g/kg, purchased from MedChemExpress (MCE)) 1 hour prior to behavioral testing.

To localize the upstream brain regions of PVN^{OXT} neurons, two recombinant adeno-associated viruses (rAAVs) were injected into the PVN of OXT-IRES-Cre mice: rAAV-EF1 α -DIO- Δ RVG-WPRE-hGH polyA (5.0×10^{12} vg/ml, PVN injection volume 200 nl) and rAAV-EF1 α -DIO-H2B-EGFP-T2A-TVA-WPRE-hGH pA (5.5×10^{12} vg/ml, PVN injection volume 200 nl). Three weeks later, RV-CVS-ENVA-N2C(Δ G)-tdTomato virus [2×10^8 IFU/ml (IFU: infectious focus unit), injection volume 100 nl] was injected into the same location in the PVN. Under fluorescence microscopy, positive neurons appeared red.

4.5 Three Chamber Social Test

Social behavior in mice was assessed using the three-chamber social test. Briefly, the experimental mice were first allowed to habituate in an empty chamber for 10 minutes. During the testing phase, the mice were then placed in a chamber containing either a novel mouse (social stimulus) or an empty cage (non-social control) and were allowed to explore for 10 minutes freely. Interaction time was recorded using a behavioral analysis system, and a social index was calculated (time spent interacting with the novel mouse/total exploration time). A reduction in this index indicates the presence of social deficits. The social preference index and social discrimination index were calculated using the following formulas: Social preference index = (Time spent with a mouse - Time spent with an empty cage)/(Time spent with a mouse + Time spent with an empty cage). Social discrimination index = (Time spent with a novel mouse - Time spent with a familiar mouse)/(Time spent with a novel mouse + Time spent with a familiar mouse)

4.6 Immunofluorescence Staining

After behavioral testing, mice were anesthetized with isoflurane and subjected to cardiac perfusion with physiological saline, followed by 4% paraformaldehyde for fixation. The brain tissue was then extracted and fixed overnight in 4% paraformaldehyde, followed by dehydration in 20% and 30% sucrose solutions until the tissue sank, ensuring cryoprotection. Coronal brain sections (25 µm thick) were obtained using a Leica cryostat, mounted on glass slides, and stored at -20°C for future use. All tissue samples for c-Fos immunofluorescence staining were collected within 1.5 hours after the completion of the behavioral experiments. Before staining, brain sections were allowed to reach room temperature and were washed with PBS. Antigen retrieval was performed using citrate buffer and microwave heating, followed by permeabilization with 0.3% Triton X-100 and blocking with BSA. Sections were then incubated overnight at 4°C with primary antibodies specific for OXT (1:1000, guinea pig, Synaptic Systems, 408004), OXTR (1:200, Rabbit, ThermoFisher, MA5-57143), c-Fos (1:500, rabbit, Cell Signaling Technology (CST), 2250) and c-Fos (1:500, guinea pig, Synaptic Systems, 226308). The following day, sections were incubated at room temperature for 2 hours in the dark with the appropriate fluorescence-conjugated secondary antibodies: Goat anti-rabbit 488 (1:500, CST, 4412S) and Goat anti-guinea pig 594 (1:1000, Jackson ImmunoResearch, JAC-106-585-003). Nuclei were counterstained with DAPI, and the sections were mounted with an anti-fade mounting medium. Fluorescent images were acquired using a fluorescence microscope for quantitative analysis.

4.7 Real-Time Quantitative PCR

Total RNA was extracted from mouse brain tissue using the TRIzol reagent method. Briefly, tissue samples were homogenized in TRIzol with the aid of grinding beads, followed by chloroform extraction and isopropanol precipitation to obtain total RNA. After washing the RNA pellet with 75% ethanol, it was dissolved in RNase-free water. RNA concentration and purity were assessed using a micro-volume spectrophotometer, with an OD260/280 ratio between 1.9 and 2.1. Subsequently, 1 µg of total RNA was reverse transcribed into cDNA using the PrimeScript RT Master Mix Kit. Real-time quantitative PCR (qPCR) was performed on a real-time fluorescence PCR instrument using a two-step program: 1 minute of pre-denaturation at 95°C, followed by 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds

(fluorescent signals were collected at this step). Primers for tested genes were designed as: OXTR forward 5'-TCTTCTTCGTGCAGATGTGG-3', reverse 5'-CCTTCAGGTACCGAGCAGAG-3'; GAPDH forward 5'-GTCTACTGGCGTCTTCAACCAC-3', Reverse: 5'-ATGACCCTCTTGGCTCCAC-3')

4.8 Immunohistochemistry

Brains from mice euthanized under deep 5% isoflurane anesthesia were fixed in 4% PFA and paraffin embedded. We cut 3- μ m sections, deparaffinized them with xylene, and dehydrated them through an ethanol series. Using a commercial IHC kit (PV-9000, ZSGB-BIO), we performed antigen retrieval and then blocked endogenous peroxidase with 5% hydrogen peroxide. Sections were incubated overnight at 4°C with an anti-OXT primary antibody (guinea pig, 1:1000; Synaptic Systems, 408004), followed by a species-matched secondary antibody. Signal was developed with DAB, and nuclei were counterstained with hematoxylin. We captured images on a Leica DM1000 microscope.

4.9 Statistical analysis

Data are presented as the mean $\pm$ SEM. All statistical analyses were performed using GraphPad Prism software (version 10.0). Depending on the experimental design, either one-way or two-way ANOVA, or unpaired Student's t-tests were applied. $P < 0.05$ was considered statistically significant.

Declarations

Data Availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. All the data related to this paper may be requested from the authors.

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Author contributions: X.M.W. designed the project. B.C.L. collected behavioral, imaging, and functional validation data. Y.D.Z. and L.L.L. performed the analysis of the data. Y. W. drafted the figures and provided a critical interpretation of the data. B.C.L. wrote the manuscript. All authors revised and approved the manuscript.

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Figures

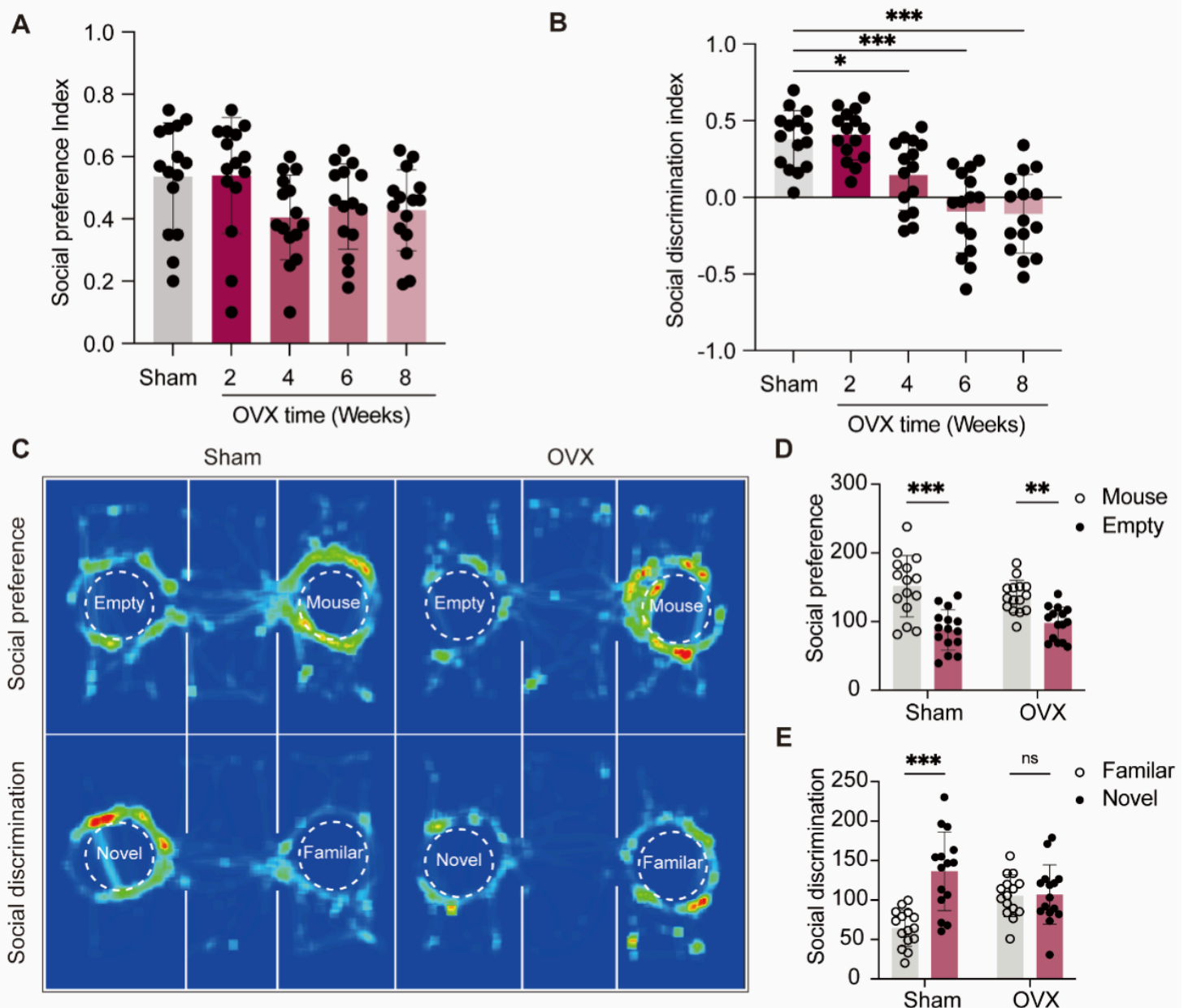


Figure 1

Estrogen withdrawal impairs social novelty in OVX mice. A. Social preference index at 2, 4, 6, and 8 weeks post-OVX. B. Social discrimination index at 2, 4, 6, and 8 weeks post-OVX (one-way ANOVA, $n = 15$). C. Heatmap of the three-chamber social test at 6 weeks post-OVX. D. Social preference in the three-chamber social test at 6 weeks post-OVX. E. Social discrimination in the three-chamber social test at 6 weeks post-OVX. For panels D and E, statistical analyses were performed using two-way ANOVA followed by Šídák's multiple comparisons test for pairwise comparisons ($n = 15$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

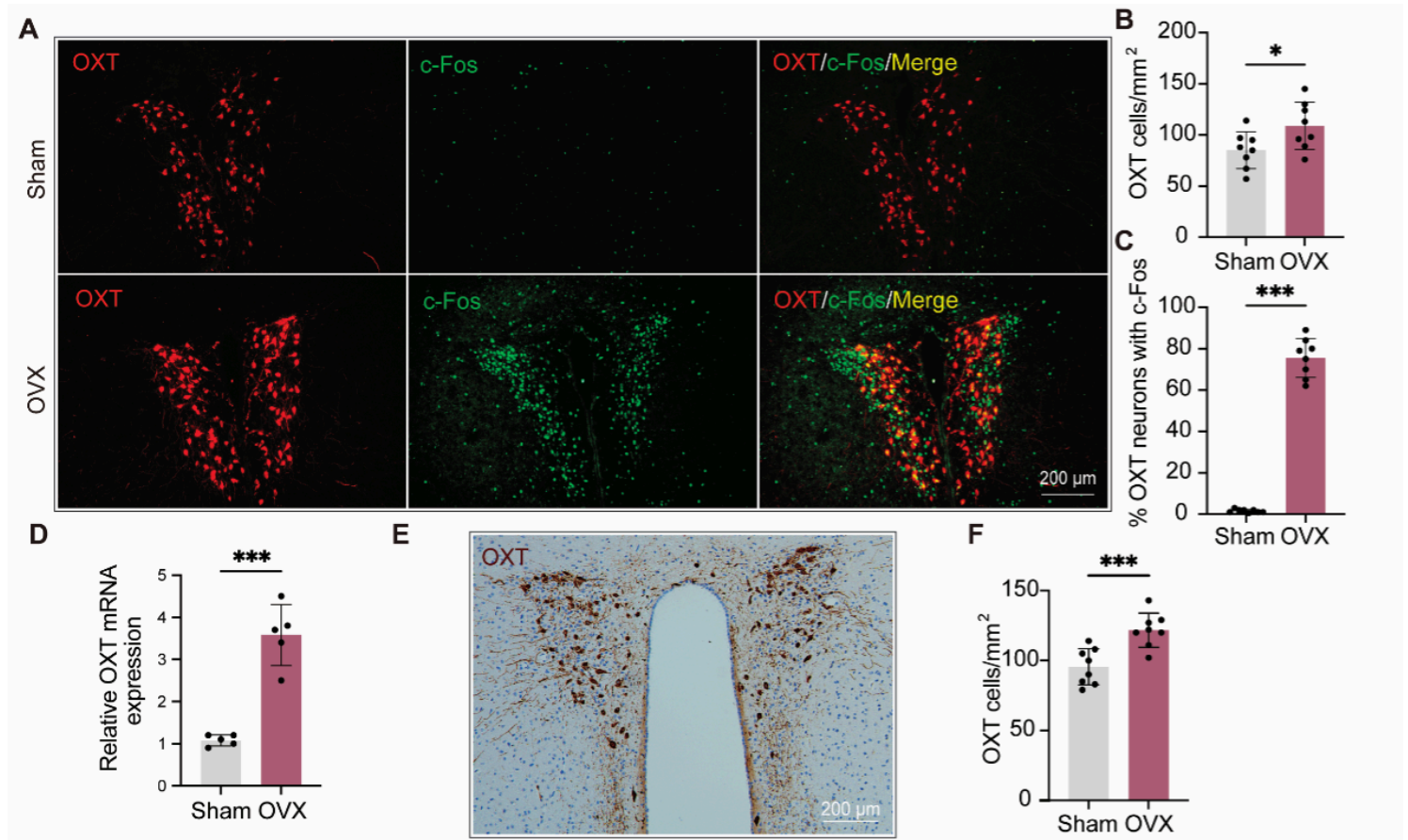


Figure 2

Effects of estrogen withdrawal on OXT neurons in female mice. A. Representative images showing c-Fos (green) co-localized with OXT (red) in the PVN. B. OXT expression in mice with estrogen withdrawal ($n = 8$). C. Representative immunofluorescence images of c-Fos (green) and OXT neurons (red) in the PVN of OVX mice (unpaired t-test, $n = 8$). D. OXT mRNA expression in the PVN of OVX mice ($n = 5$). E. Representative images of OXT-immuno-stained neurons in the PVN. F. Quantification of OXT-positive neurons in the PVN after OVX ($n = 8$). All statistical analyses were performed using unpaired t-tests ($*P < 0.05$, $***P < 0.001$).

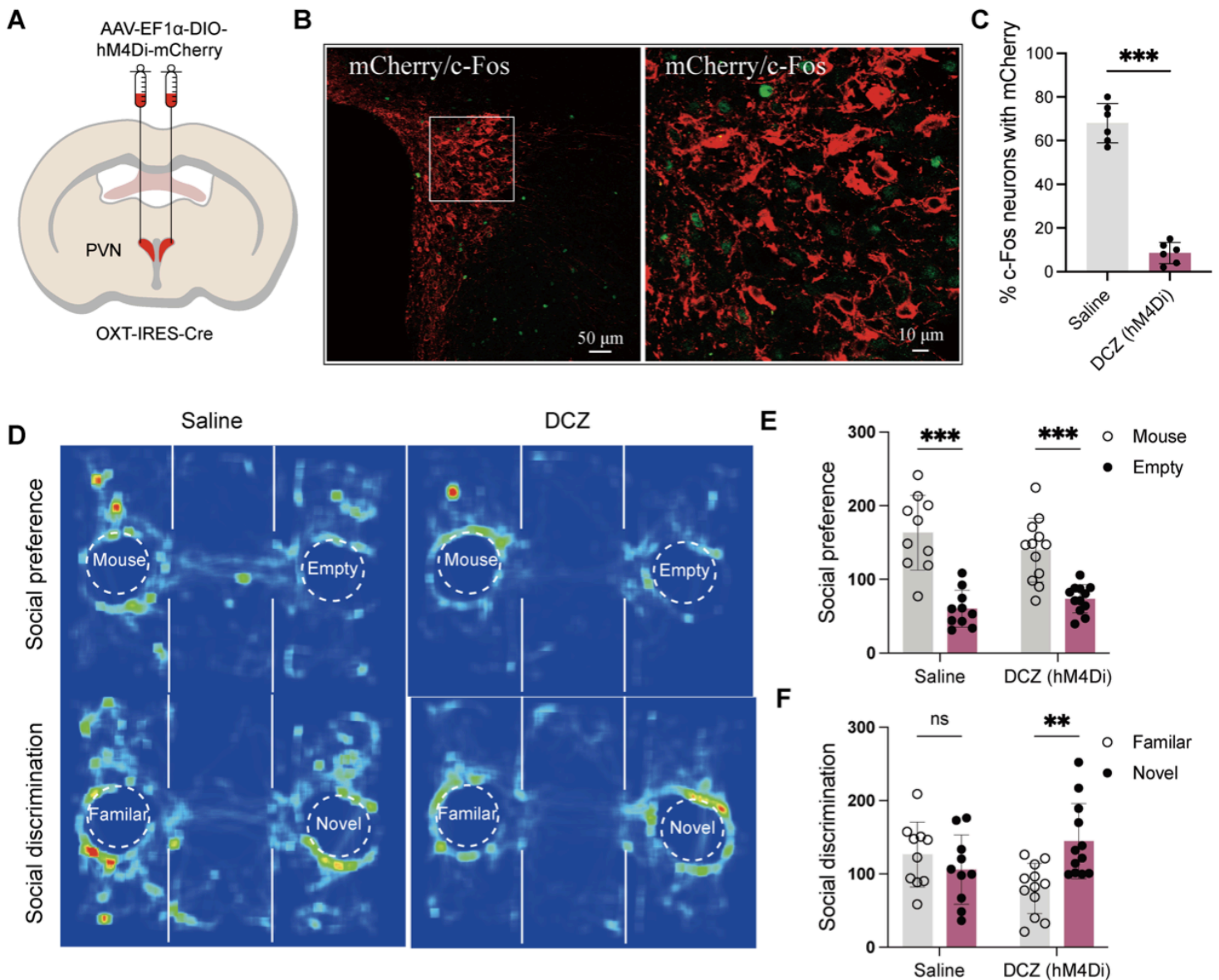


Figure 3

Chemogenetic inhibition of PVN^{OXT} neurons enhances social memory in OVX mice. A. Schematic diagram of chemogenetic inhibition of PVN^{OXT} neurons in OVX mice. B. Representative immunofluorescence images of c-Fos (green) in mCherry-positive PVN^{OXT} neurons (red) following saline or DCZ treatment in OXT-IRES-Cre mice. C. Quantification of c-Fos⁺/mCherry⁺ double-positive neurons in the PVN (unpaired t-test, *** $P < 0.001$, $n = 6$). D. Heatmap of the three-chamber social test. E. Social preference in the three-chamber social test: saline group (mouse vs. empty), *** $P < 0.001$; DCZ (hM4Di) group (mouse vs. empty), Šídák's multiple comparisons test, *** $P < 0.001$. F. Social discrimination in the three-chamber social test: saline group (familiar vs. novel mouse), Šídák's multiple comparisons test, $P = 0.5193$; DCZ (hM4Di) group (familiar vs. novel mouse), Šídák's multiple comparisons test, ** $P = 0.002$. All statistical analyses were performed using two-way ANOVA (saline group, $n = 10$; DCZ (hM4Di) group, $n = 12$).

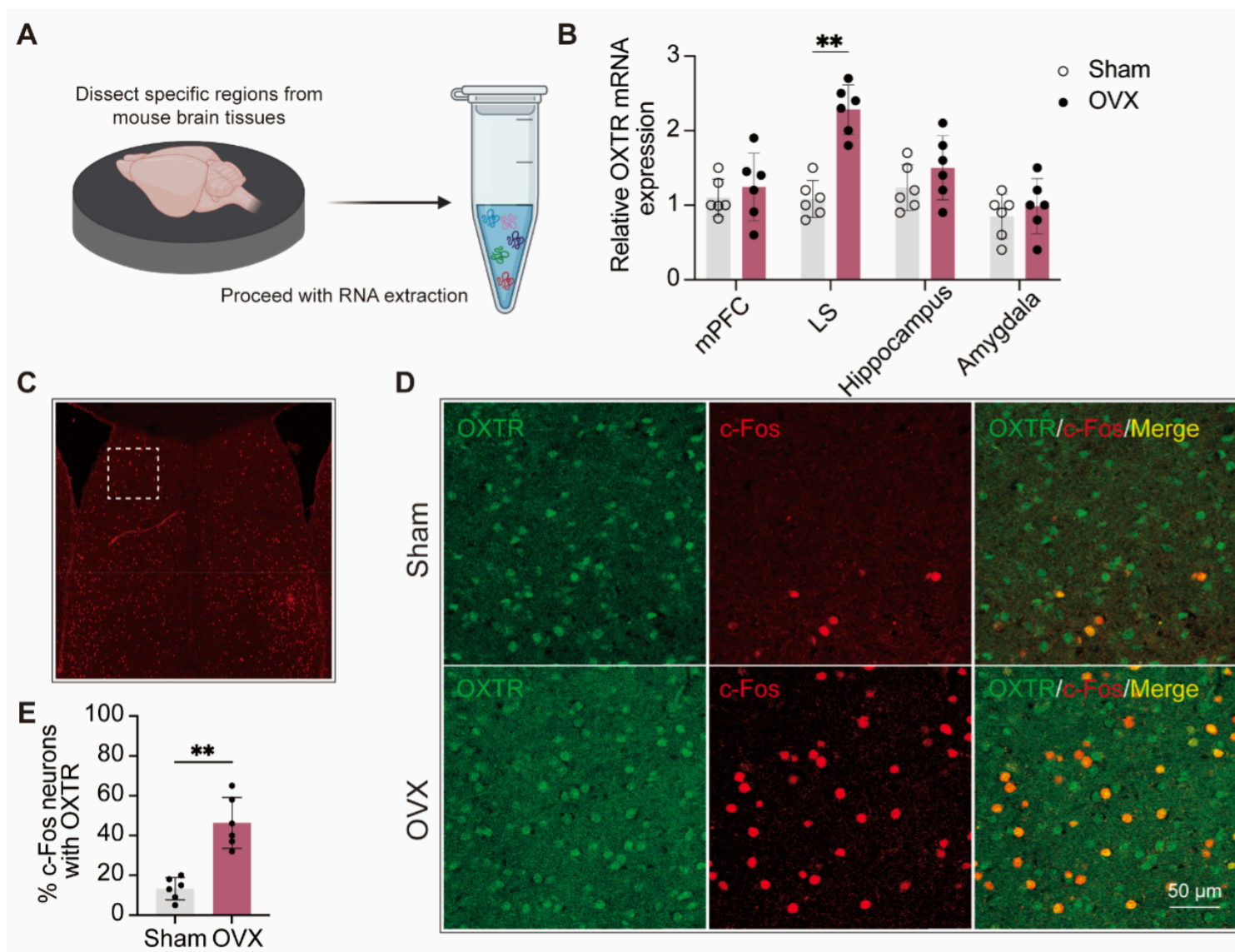


Figure 4

Effect of estrogen withdrawal on OTR density in OXT-sensitive PVN afferents. A. Schematic diagram of work model. B. OXTR mRNA expression (two-way ANOVA followed by Šídák's multiple comparisons test, $**P = 0.0034$, $n = 6$). C. Schematic diagram of the LS. D. Representative immunofluorescence images showing c-Fos (Red) in OXTR⁺ neurons (Green) in the LS. E. Quantification of c-Fos⁺/OXTR⁺ double-positive neurons in the LS.

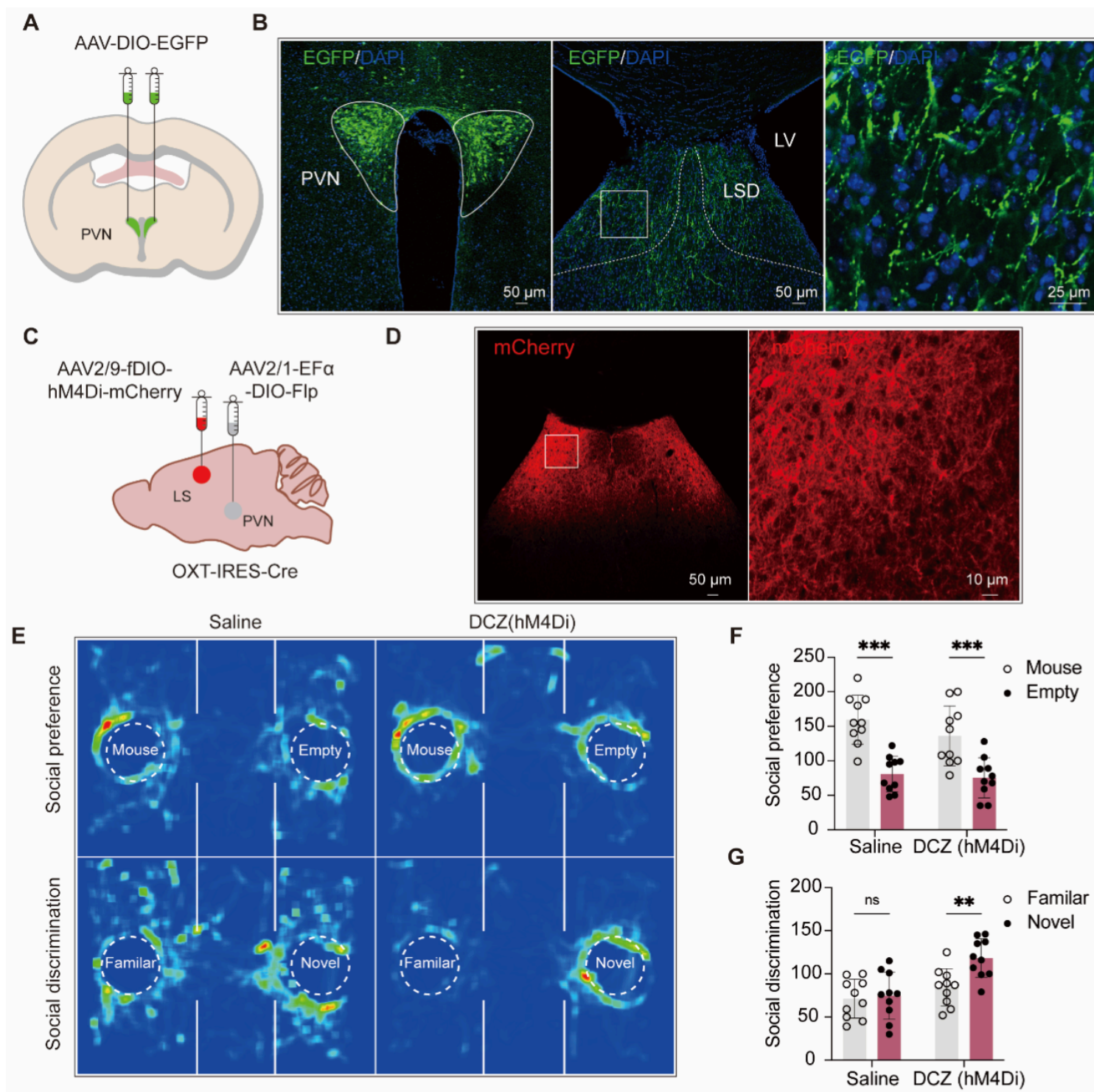


Figure 5

Chemogenetic inhibition of LS neurons innervated by PVN^{OXT} neurons enhances social memory in OVX mice. A. Schematic diagram of AAV-DIO-EGFP in the PVN. B Representative OXT neurons in the PVN (left), and the downstream of PVN^{OXT} neurons in the LS (right). C. Schematic diagram of inhibition of LS neurons receive projection of PVN^{OXT}. D. Representative immunofluorescence images of mCherry-positive neurons (red) innervated by PVN^{OXT} neurons following saline or DCZ treatment in OXT-IRES-Cre mice. E. Heatmap of the three-chamber social test. F. Social preference in the three-chamber social test:

saline group (mouse vs. empty), $***P < 0.001$; DCZ (hM4Di) group (mouse vs. empty), Šídák's multiple comparisons test, $***P = 0.0006$, $n = 10$. G. Social discrimination in the three-chamber social test: saline group (familiar vs. novel mouse), Šídák's multiple comparisons test: saline group (familiar vs. novel mouse), Šídák's multiple comparisons test, $P = 0.9335$; DCZ (hM4Di) group (familiar vs. novel mouse), Šídák's multiple comparisons test, $**P = 0.0055$.

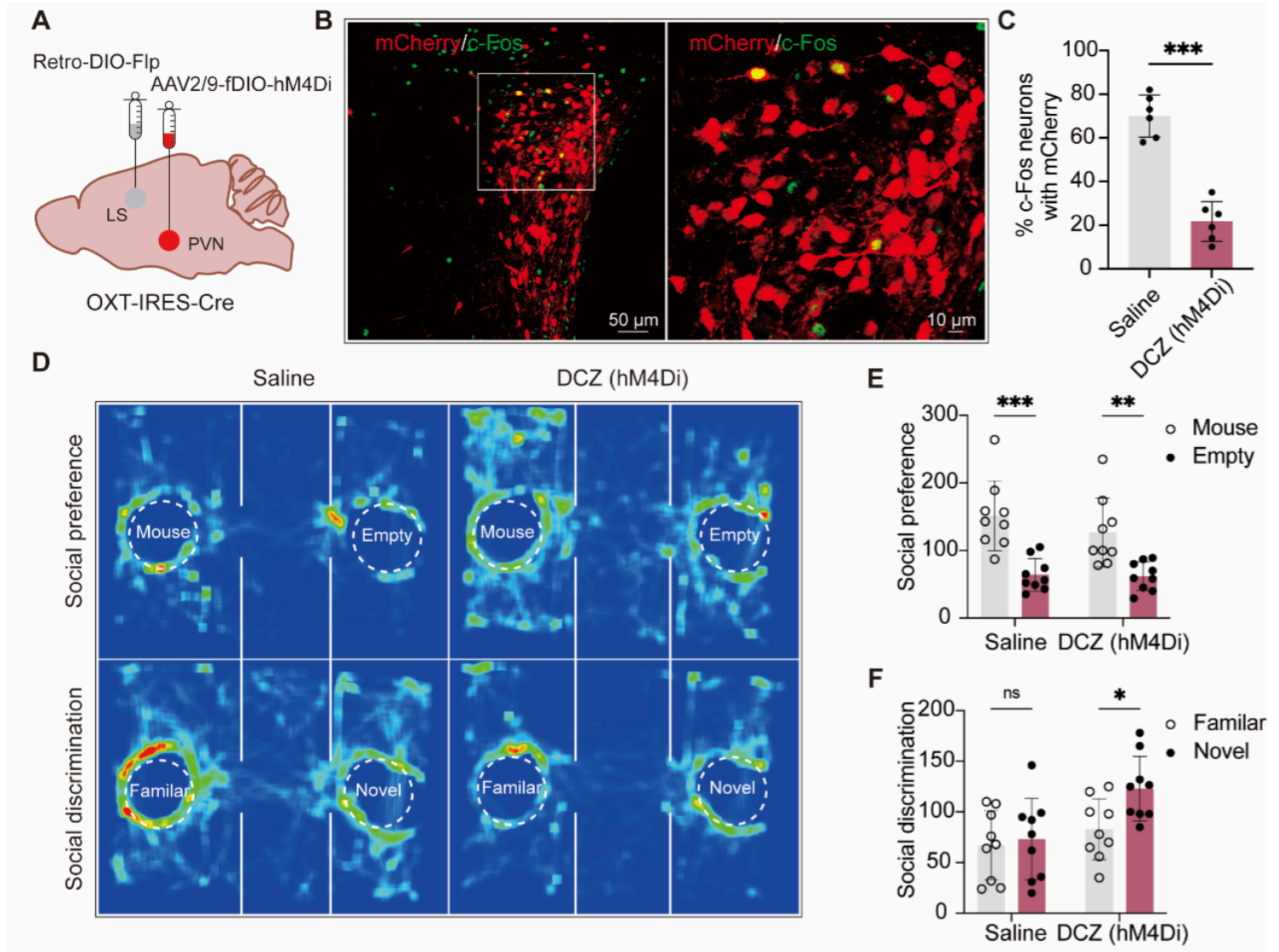


Figure 6

Chemogenetic inhibition of PVN^{OXT} neurons projecting to LS improves social behavior in OVX mice. A. Schematic diagram of inhibition of PVN^{OXT} neurons projecting to LS. B. Representative immunofluorescence images of c-Fos (green) in mCherry-positive neurons (red) in the PVN. C. Quantification of c-Fos⁺/mCherry⁺ double-positive neurons in the PVN (unpaired t-test, $***P < 0.001$, $n = 6$). D. Heatmap of the three-chamber social test. E. Social preference in the three-chamber social test: saline group (mouse vs. empty), $***P = 0.001$; DCZ (hM4Di) group (mouse vs. empty), Šídák's multiple comparisons test, $**P = 0.0031$, $n = 9$. F. Social discrimination in the three-chamber social test: saline group (familiar vs. novel mouse), Šídák's multiple comparisons test: saline group (familiar vs. novel

mouse), Šídák's multiple comparisons test, $P = 0.9146$; DCZ (hM4Di) group (familiar vs. novel mouse), Šídák's multiple comparisons test, $*P = 0.0369$.

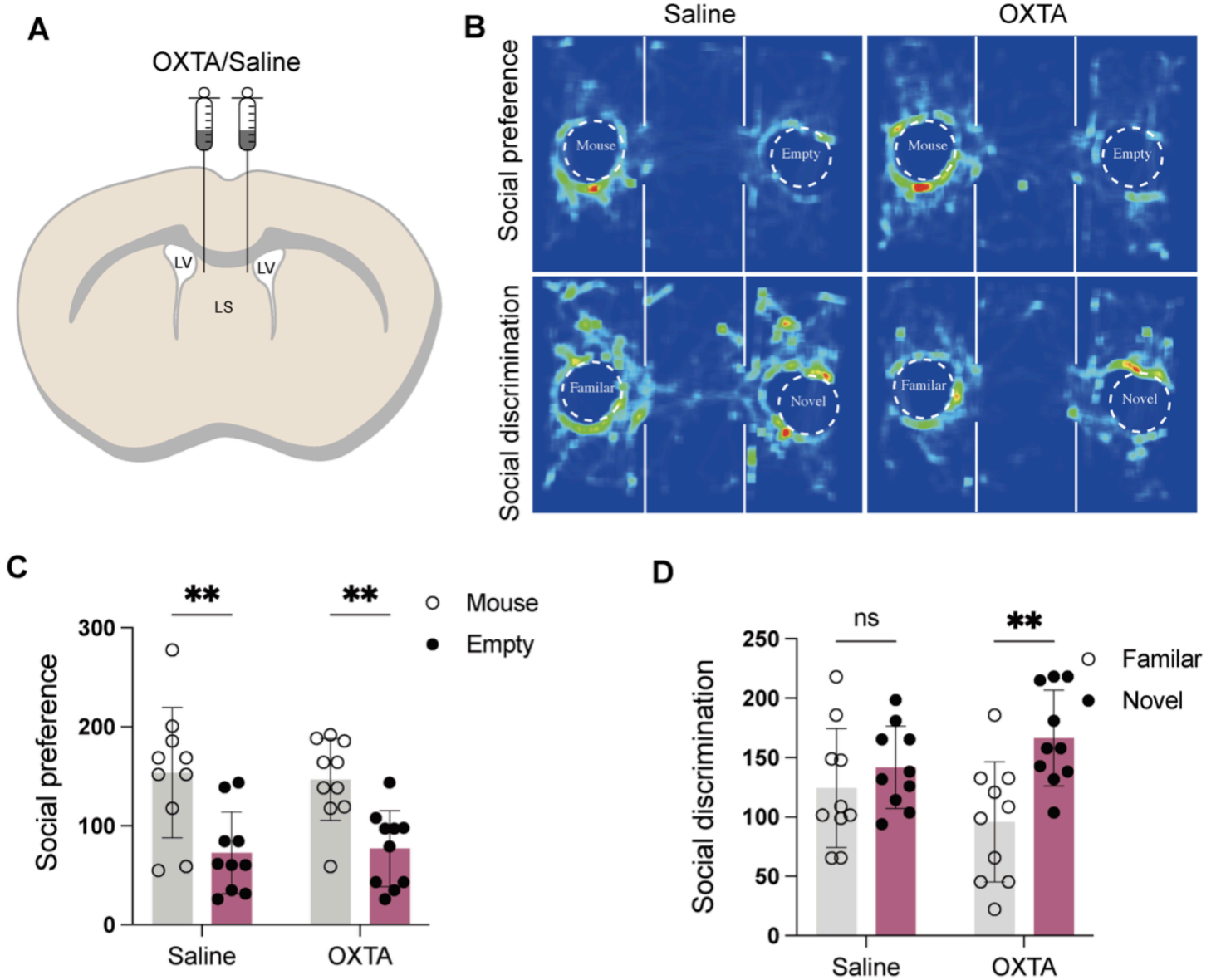


Figure 7

Effect of inhibiting OTRs in the LS during estrogen withdrawal. A. Schematic diagram of injection of OXT antagonist in the LS. B. Heatmap of the three-chamber social test. C. Social preference in the three-chamber social test: saline group (mouse vs. empty), $**P = 0.0012$; OXTA group (mouse vs. empty), Šídák's multiple comparisons test, $**P = 0.0051$, $n = 10$. D. Social discrimination in the three-chamber social test: saline group (familiar vs. novel mouse), Šídák's multiple comparisons test: saline group (familiar vs. novel mouse), Šídák's multiple comparisons test, $P = 0.6193$; OXTA group (familiar vs. novel mouse), Šídák's multiple comparisons test, $**P = 0.0021$.

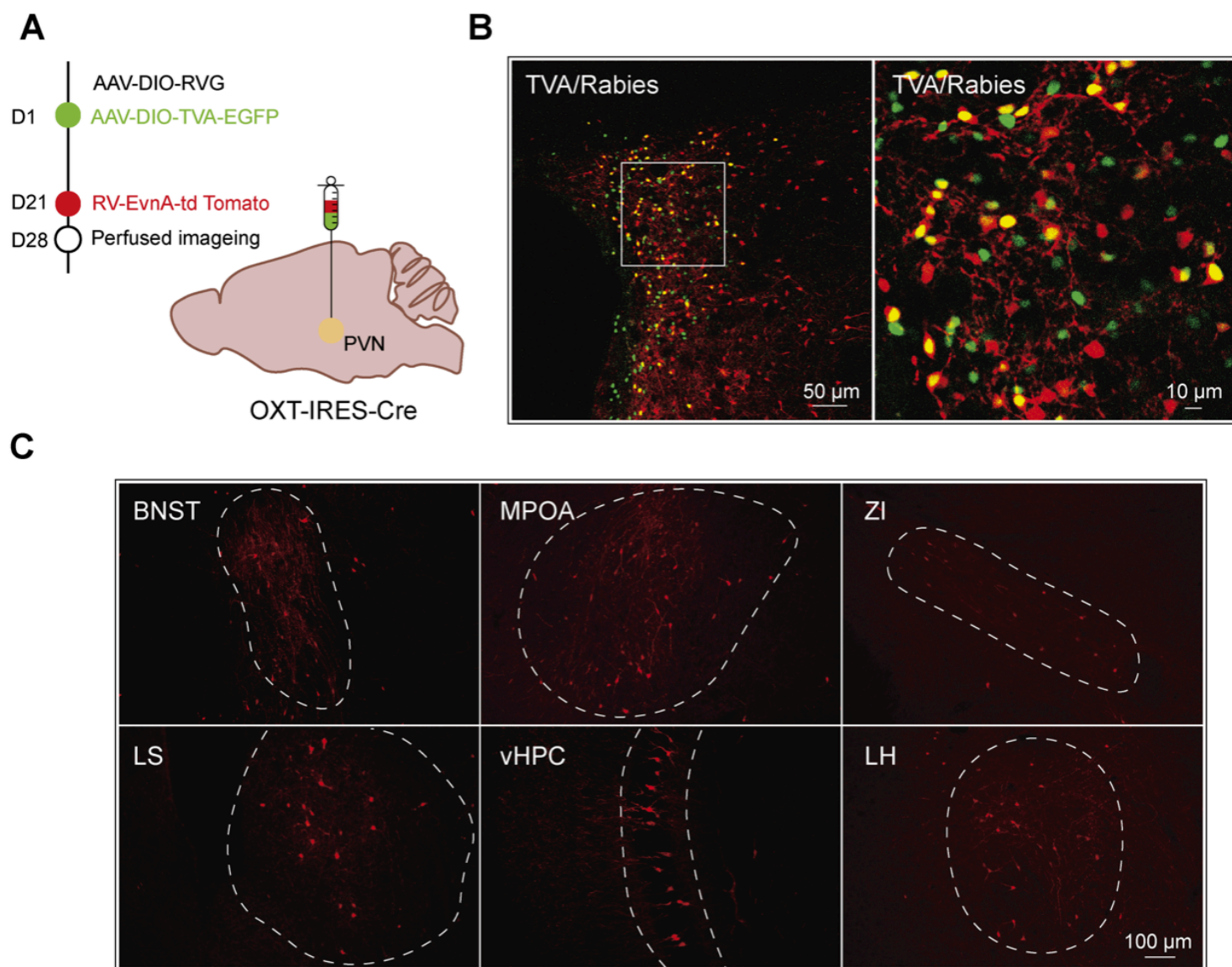


Figure 8

Monosynaptic inputs to PVN^{OXT} neurons (Retrograde tracing). A. Schematic illustration of the injection protocol. B. The rabies-tdTomato signal was colocalized with TVA-GFP immunofluorescence in the PVN, confirming successful trans-synaptic labeling of neurons projecting to the PVN^{OXT} neurons. C. Representative retrogradely labeled neurons in various brain regions: BNST, MPOA, ZI, LS, HPC, and LH; BNST, bed nucleus of the stria terminalis; MPOA, medial preoptic area; ZI, zona incerta; LS, lateral septum; vHPC, ventral hippocampus; LH, lateral hypothalamic.

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