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Supplemental Information

Ventral Hippocampal-Prefrontal Interaction Affects Social Behavior via Parvalbumin Positive Neurons in the Medial Prefrontal Cortex

Qingtao Sun, Xiangning Li, Anan Li, Jianping Zhang, Zhangheng Ding, Hui Gong, and Qingming Luo

TRANSPARENT METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-PV	Millipore	MAB1572
anti-SST	santa cruz	sc-7819
anti-VIP	Millipore	AB982
Alexa Fluor 405,Gt-Anti-Mouse,H;	Invitrogen	A-31553
Alexa Fluor 647,Rb-Anti-Gt ,H+L;	Invitrogen	A-21446
Alexa Fluor 405,Gt-Anti-Rabbit,H.	Invitrogen	A-31556
Bacterial and Virus Strains		
AAV8-CAG -FLEx-glycoprotein	UNC Vector Core (Chapel Hill, NC)	Naoshige Uchida
AAV8- EF1a -FLEx-TVA-mCherry	UNC Vector Core (Chapel Hill, NC)	Naoshige Uchida
AAV2/9-EF1a-DIO-hCHR2(H134R)-mCherry	BrainVTA Co., Ltd., Wuhan, China	PT-0002
AAV2/9-EF1a-DIO-GCaMP6s	BrainVTA Co., Ltd., Wuhan, China	PT-0071
AAV2/9-CaMKII-NpHR3.0-YFP	BrainVTA Co., Ltd., Wuhan, China	PT-0008
SAD-ΔG-GFP(EnvA)-RV	BrainVTA Co., Ltd., Wuhan, China	R01001
SAD-ΔG-DsRed(EnvA)-RV	BrainVTA Co., Ltd., Wuhan, China	R01002
AAV2/9-EF1a-DIO-NpHR3.0-mCherry	BrainVTA Co., Ltd., Wuhan, China	PT-0007
RetroAAV-Cre	BrainVTA Co., Ltd., Wuhan, China	PT0136
AAV2/9- EF1a-fDIO-hChR2-mCherry	Taitool Bioscience Co. Ltd, Shanghai, China	S0404
AAV2/9- EF1a-DIO-hM4D(Gi)-mCherry	Taitool Bioscience Co. Ltd, Shanghai, China	S0193
AAV2/9- CaMKII-hM4D(Gi)-mCherry	Taitool Bioscience Co. Ltd, Shanghai, China	S0194
Chemicals, Peptides, and Recombinant Proteins		
PBS	Sigma-Aldrich	P3563
paraformaldehyde	Sigma-Aldrich	158127
Sucrose	Sigma-Aldrich	V900116
clozapine-n-oxide (CNO)	enzo lifesciences	BML-NS105-0005
Experimental Models: Organisms/Strains		
PV-Cre	Jackson Laboratory	008069
SST-Cre	Jackson Laboratory	013044
VIP-Cre	Jackson Laboratory	010908
PV-2A-Flpo	Jackson Laboratory	022730
C57BL/6	Hfkbio Co. Ltd, Beijing, China	
Software and Algorithms		

MATLAB	Mathworks, Inc.	https://www.mathworks.com
Graphpad prism 6.01	GraphPad Software	https://www.graphpad.com

CONTACT FOR REAGENT AND RESOURCE SHARING

All requests for resources should be directed to and will be fulfilled by Qingming Luo (qluo@mail.hust.edu.cn)

EXPERIMENTAL MODEL AND SUBJECT DETAILS

ANIMALS

PV-Cre (Madisen et al., 2010), SST-Cre, VIP-Cre (Taniguchi et al., 2011), PV-flpo (Madisen et al., 2015) and C57BL/6 adult male mice (2-3months) were used for virus tracing, optogenetics, chemogenetics and fiber photometry. In social discrimination test, juvenile male mice (<8 weeks) were used as novel targets. Mice were housed under 22 ± 1 °C and 55 ± 5% humidity with food and water ad libitum. Animal experiments were conducted in accordance with the Institutional Animal Ethics Committee of Huazhong University of Science and Technology.

VIRUS

The AAV8-CAG -FLEx-glycoprotein (Watabe-Uchida et al., 2012) (3.3×10^{12} gc/ml) and AAV8- EF1a -FLEx-TVA-mCherry (Watabe-Uchida et al., 2012) (8×10^{12} gc/ml) were purchased from the UNC Vector Core (Chapel Hill, NC). AAV2/9-EF1a-DIO-hCHR2(H134R)-mCherry(2×10^{12} gc/ml), AAV2/9-EF1a-DIO-GCaMP6s(2×10^{12} gc/ml), AAV2/9-CaMKII-NpHR3.0-YFP (2×10^{12} gc/ml), SAD-ΔG-GFP(EnvA)-RV(5×10^{8} IU/mL) and SAD-ΔG-DsRed(EnvA)-RV (5×10^{8} IU/mL), AAV2/9-EF1a-DIO-NpHR3.0-mCherry(2×10^{12} gc/ml), RetroAAV-Cre(5×10^{12} gc/ml) were purchased from BrainVTA (BrainVTA Co., Ltd., Wuhan, China). AAV2/9- EF1a-fDIO-hChR2-mCherry (2×10^{12} gc/ml), AAV2/9- EF1a-DIO-hM4D (Gi) -mCherry (2×10^{12} gc/ml), AAV2/9- CaMKII-hM4D(Gi)-mCherry(2×10^{12}gc/ml) were purchased from Taitool Bioscience (Taitool Bioscience Co. Ltd, Shanghai, China).

VIRUS INJECTIONS

For retrograde monosynaptic tracing, 150 nL viral cocktail(1:2) containing AAV8-EF1a -FLEx-TVA-mCherry and AAV8-CAG -FLEx-glycoprotein was injected into the prelimbic area(bregma1.9 mm, lateral 0.3 mm, depth 2.3 mm from skull surface) of PV-

Cre, SST-Cre and VIP-Cre mice. Three weeks later, 300-400 nL SAD-ΔG-GFP(EnvA)-RV or SAD-ΔG-DsRed(EnvA)-RV was injected into the same site.

For activation of GABAergic neurons in the mPFC, 300 nL AAV2/9-EF1a-DIO-hCHR2(H134R)-mCherry was injected into prelimbic area. For inhibition of vHIP, 400nl AAV2/9-CaMKII-NpHR3.0-YFP was injected into vHIP bilaterally (bregma-3.4mm, lateral ±3.5 mm, depth 4 mm from skull surface).

For fiber photometry, 300 nL AAV2/9-EF1a-DIO-GCaMP6s was injected into prelimbic area. The optical fiber (200 mm O.D., 0.37 numerical aperture (NA), Newdoon Inc. China;) was placed 300 μm above the virus injection sites. All the viruses were delivered by a sharp micropipette mounted on a Nanoject II(Drummond Scientific Co., Broomall, PA, USA) attached to a micromanipulator and then injected at a speed of 60 nL per min. The glass micropipette was held for an extra 10 min after the completion of the injection and then slowly retreated. After the surgery, the incisions were stitched and lincomycin hydrochloride and lidocaine hydrochloride gel was applied to prevent inflammation and alleviate pain for the animals. For optogenetics and fiber photometry, dental cement was used to fix the optical fibers.

For chemogenetics, 300 nL AAV2/9- EF1a-DIO-hM4D(Gi)-mCherry was injected into the vHIP of PV-flpo mice bilaterally, 300 nL viral cocktail (1:2) containing RetroAAV-Cre and AAV2/9- EF1a-fDIO-hChR2-mCherry was injected into the prelimbic area simultaneously. 300 nL AAV2/9- CaMKII-hM4D(Gi)-mCherry was injected into the vHIP of VIP-Cre mice bilaterally, 300 nLAAV2/9-EF1a-DIO-NpHR3.0-mCherry was injected into the prelimbic area simultaneously. After the virus injections, the optical fibers was planted into the prelimbic area with dental cement bilaterally for optogenetics manipulations.

HISTOLOGY

Mice were deeply anesthetized with sodium pentobarbital (1% wt/vol) and subsequently intracardially perfused with 0.01M PBS (Sigma-Aldrich Inc., St Louis, MO, USA), followed by 4% paraformaldehyde (Sigma-Aldrich Inc., St Louis, MO, USA) and 2.5% sucrose (Sigma-Aldrich Inc., St Louis, MO, USA) in 0.01M PBS. The brains were excised and post-fixed in 4% paraformaldehyde at 4°C for 12 h. For immunohistochemistry, the mouse brain was sectioned at 50μm on a vibration microtome (Leica, VT1200S). The sections of interests were blocked with 5%(wt/vol) BSA containing 0.3% Triton-X 100 (vol/vol) in 0.01 M PBS for 1 h, then incubated with the following primary antibodies (12 h at 4 °C):anti-PV(1:1000, mouse,

Millipore,MAB1572), anti-SST(1:200, goat, santa cruz, sc-7819), anti-VIP(1:100, rabbit, AB982). After rinsing, sections were incubated with following fluorophore-conjugated secondary antibody for 2 h at room temperature (1:400; Invitrogen): Alexa Fluor 405,Gt-Anti-Mouse,H; Alexa Fluor 647,Rb-Anti-Gt ,H+L; Alexa Fluor 405,Gt-Anti-Rabbit,H. Antibodies were diluted in the same block solution.

MICROSCOPY

For immunohistochemistry imaging, the sections were mounted by 50% glycerol (vol/vol) and imaged using a 20×, 0.75NA objective (Zeiss 710). For dual color RV imaging shown in Fig.1, the sections were mounted by 50% glycerol (vol/vol) and imaged using a 10×, 0.45NA objective (Olympus VS120 virtual microscopy slide scanning system, Olympus, Shanghai, China). For hippocampal axon terminal imaging, the brain slices containing mPFC area were imaged using a 40×, 1.4NA oil objective (Zeiss 710).

METHOD DETAILS

SOCIAL DISCRIMINATION TEST

The social discrimination test (SDT) procedures were similar to previous studies (Okuyama et al., 2016). Test mice were handled by the investigator for several minutes on each of two separate days (Day-1 and Day-2). The test mice were allowed to explore the social discrimination chamber (40 x 40 cm square, 30cm height) for ten minutes on Day-1 and Day-2 with pencil holders (circle with a radius of 7.5 cm and 15 cm height) placed at the upper right and lower left corners of social discrimination chamber. On day-3, the test mice were placed in social discrimination chamber, while the littermates and novel mice were kept in the pencil holders placed at the upper right and lower left corners of social discrimination chamber. The test mice were allowed to explore the chamber for 4 minutes before returned to home cages. Behavioral recordings and trackings were performed for 4 min by Ethovision XT software, using an infra-red (IR) sensitive GIG-E camera with two IR illuminators under dark conditions. On day-4, we used 570nm laser (20mW, persistent inhibition) to inhibit the activity of vHIP through optical fibers (Intelligent light system, Newdoon Inc. China) and repeated the same experimental procedure on Day-3. On Day-5, we used 470nm laser (15mW, 20Hz,15ms duration) to activate the GABAergic neurons in the mPFC and 570nm laser to inhibit the activity of vHIP simultaneously through optical fibers (Intelligent light system, Newdoon Inc. China) and repeated the same experimental procedure on Day-3. All the

light delivery were 1min later after the test mice entered the social discrimination chamber and last for 3 min. During SDT on day-3, day-4 and day-5, different novel mice was used to avoid the familiarization between the test mice and stimulus mice. The social interactions were manually scored when the test mice sniffed or climbed the pencil holders at the corners of social discrimination chamber while the travelling distance and speed were automatically calculated by Ethovision XT software.

For acute chemogenetics manipulations, PV-flpo mice and VIP-Cre mice that expressed hM4Di in their hippocampus were acclimated to IP needle pokes for one weeks prior to behavioral tests, though were not injected with any substance. On testing day, mice received a single IP injection of CNO (5mg/kg of body weight) 30min before testing. CNO was was dissolved in 0.9%(wt/vol) saline (5mg in 10ml). The control group received a single IP injection of 0.9%(wt/vol) saline instead of CNO. For blank control group, C57BL/6 mice were acclimated to IP needle pokes for one weeks prior to behavioral tests, though were not injected with any substance. On testing day, C57BL/6 mice received a single IP injection of CNO (5mg/kg of body weight) 30min before testing. After the behavior test, the mice were allowed to rest for 1h. Then the PV-flpo mice and VIP-Cre mice that received CNO injections were tested again. Different novel mice was used to avoid the familiarization between the test mice and stimulus mice. In the meantime, we used 470nm laser (15mW, 20Hz,15ms duration) to activate the $PV+_{mPFC}$ of PV-flpo mice and 570nm laser (20mW, persistent inhibition) to inhibit the $VIP+_{mPFC}$ (VIP+ neurons in mPFC) of VIP-Cre mice.

NOVEL/FAMILIAR OBJECT TEST

Mouse handling and habituation procedures were identical to SDT. On Day-1 and Day-2, The test mice were allowed to explore the chamber (40 x 40 cm square, 30cm height) for ten minutes with two table tennis ball (familiar object) put at the upper right and lower left corners of the chamber. On Day-3, the test mice were allowed to explore the chamber for five minutes with two table tennis ball (familiar object) put at the upper right and lower left corners of the chamber. Behavioral recording and tracking were performed for 5 min by Ethovision XT software, using an infra-red (IR) sensitive GIG-E camera with two IR illuminators under dark conditions. After the exploration, the test mice were returned to home cages to rest for ten minutes. Then the test mice explored the chamber again. The table tennis ball put at the upper right corners of the chamber was replaced by a small plastic cup (circle with a radius of 3.5 cm and 5 cm height) as novel object. The test mice were allowed to explore the chamber for five minutes before

returned to home cages. During the exploration, half of the test-mice were assessed under OFF-laser conditions while the other half received a counterbalanced protocol. Behavior recording conditions and laser conditions were identical to SDT. The object interactions were manually scored when the test mice sniffed or climbed the object at the corners of the chamber.

FIBER PHOTOMETRY

The fiber photometry procedures were similar to previous studies (Li et al., 2016, Wang et al., 2017). Fiber recordings were performed in freely moving mice three weeks after virus injection. To induce fluorescence signals, a laser beam from a laser tube (488 nm) was reflected by a dichroic mirror, focused by a 10x len (NA = 0.3) and then coupled to an optical commutator. A 3-m optical fiber (200 mm O.D., NA = 0.37) guided the light between the commutator and the implanted optical fiber. To minimize photo bleaching, the power intensity at the fiber tip was adjusted to 0.02 mW. The GCaMP6s fluorescence was band-pass filtered (MF525-39, Thorlabs) and collected by a photomultiplier tube (R3896, Hamamatsu). An amplifier (C7319, Hamamatsu) was used to convert the photomultiplier tube current output to voltage signals, which was further filtered through a low-pass filter (40 Hz cut-off; Brownlee 440). The analog voltage signals were digitalized at 100 Hz and recorded by a Power 1401 digitizer and Spike2 software (CED, Cambridge, UK). The calcium signals and the social interactions was simultaneously recorded by data acquisition software (Thinkertech, China) and the calcium data from each continuous experimental trial was normalized to the averaged fluorescence by a self-developed MATLAB program.

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical graphs were generated using Graphpad prism 6.01. The two-tailed paired, unpaired student's t test and one-way ANOVA were also performed using Graphpad prism 6.01. The AUC (area under curve) analysis was also performed using Graphpad prism 6.01. The confidence level was set to 0.05 (P value), and all results are presented as the means±s.e.m.

Reference:

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B., Nguyen, T. N., Sunkin, S. M., Benucci, A., Nagy, A., Miyawaki, A., Helmchen, F., Empson, R. M., Knopfel, T., Boyden, E. S., Reid, R. C., Carandini, M. & Zeng, H. 2015. Transgenic mice for intersectional targeting of neural sensors and effectors with high specificity and performance. *Neuron,* 85**,** 942-958.

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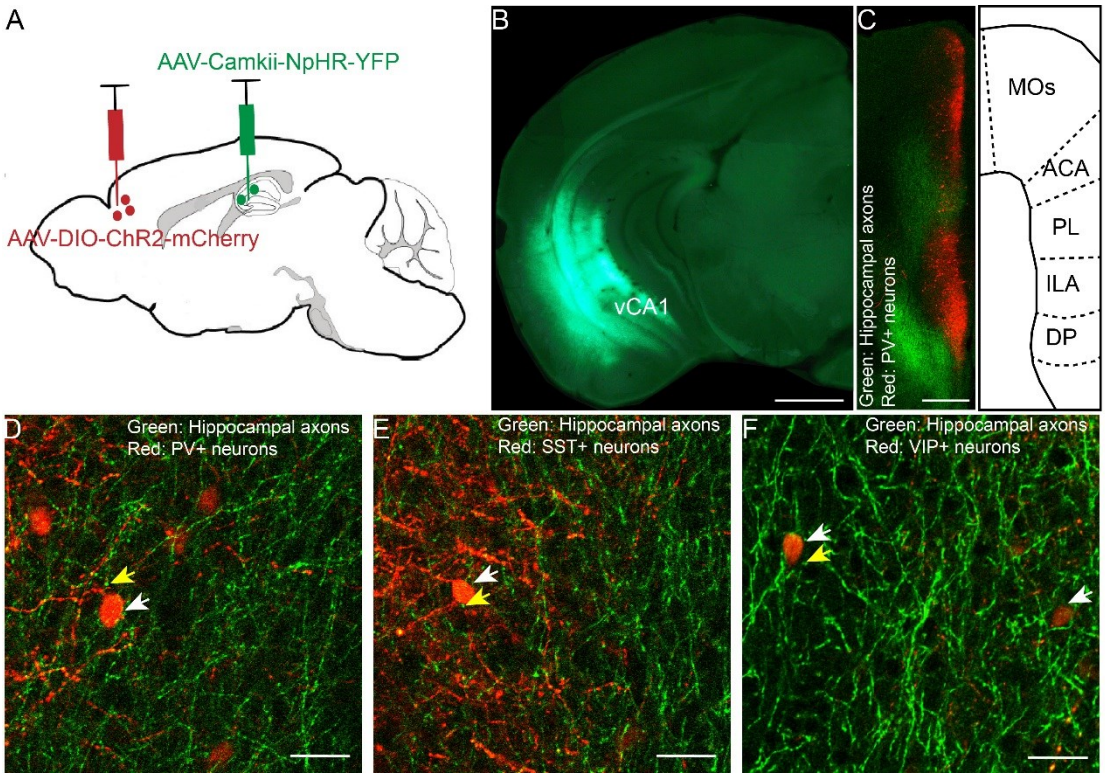
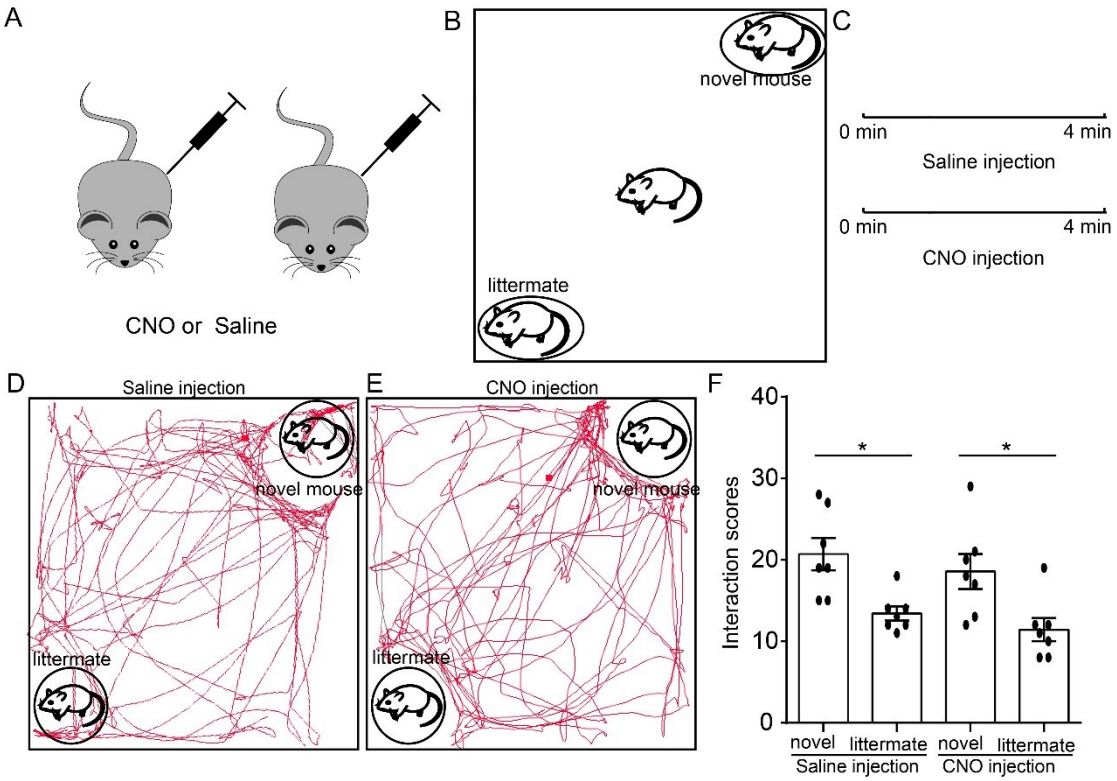
Supplementary Figure



Supplementary Figure 1 Different GABAergic neurons in mPFC are innervated by hippocampal axons, related to figure 1.

A. Schematic of the experimental strategy to label different GABAergic neurons and hippocampal axons in mPFC. AAV-DIO-ChR2-mCherry was injected into the mPFC of different Cre driver lines to label different GABAergic neurons while the AAV-Camkii-NpHR-YFP was injected into the ventral hippocampus to label the hippocampal axons in mPFC. B,C. Injection sites at the ventral hippocampus and mPFC. D-F. Different GABAergic neurons in mPFC are heavily innervated by hippocampal axons. The white arrows showed the GABAergic neurons in mPFC that are surrounded by hippocampal axons. The yellow arrows showed the boutons of hippocampal axons. Scale bar in B is 1mm. Scale bar in C is 500μm. Scale bars in D-F are 30μm.



Supplementary Figure 2 Administration of CNO alone did not affect social behavior, related to figure 5 and 6.

A. C57BL/6 mice was injected intraperitoneally with CNO or saline. B. Experimental strategy of social discrimination test. C. Timeline of social discrimination test. D, E. Position tracking from representative mice during social discrimination test with CNO injection or saline injection. F. Social interaction times with novel mice or littermates with different experimental conditions.(Paired t-test, *$p<0.05$)