

Expanded View Figures

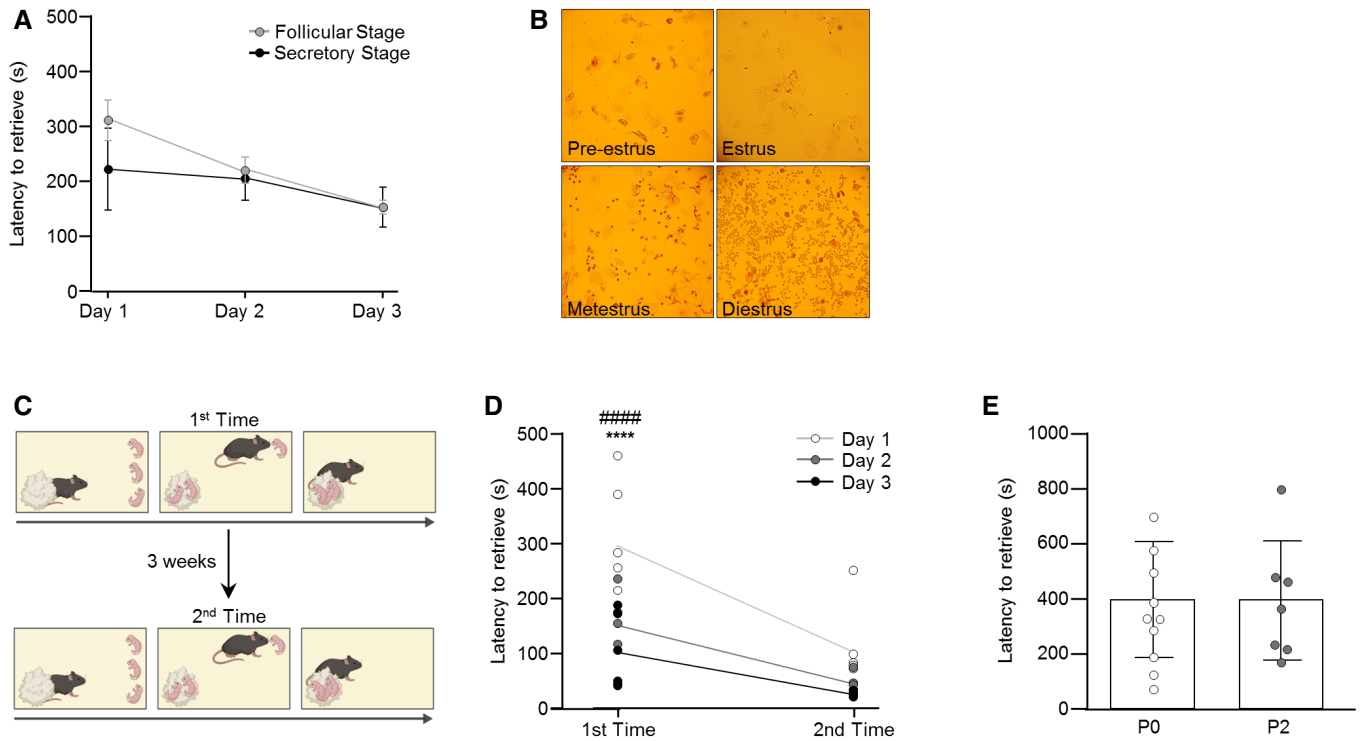


Figure EV1. Pup retrieval behavior in virgin female mice.

- A Total pup retrieval time of virgin females in the follicular or secretory stage of the estrus cycle ($n = 4/\text{group}$). Data were analyzed using two-way ANOVA with repeated measures and are expressed as mean \pm s.e.m.
- B Representative images of vaginal smears of pre-estrus, estrus, metestrus, and diestrus stage.
- C Schematic representation of the pup retrieval test at different time points.
- D Total pup retrieval time in the first and second rounds of behavioral testing in virgin female mice ($n = 6/\text{group}$). Data were analyzed using two-way ANOVA with repeated measures and are expressed as mean \pm s.e.m. ****significantly different from foster mothers, $P < 0.0001$; #####significantly different from mothers, $P < 0.0001$.
- E Total pup retrieval time in virgin females exposed to pups at different ages (P0 and P2) on the first day of pup exposure (P0: $n = 10$; P2: $n = 7$). Data were analyzed using Student's t -test and displayed as individual values \pm s.e.m.

Source data are available online for this figure.

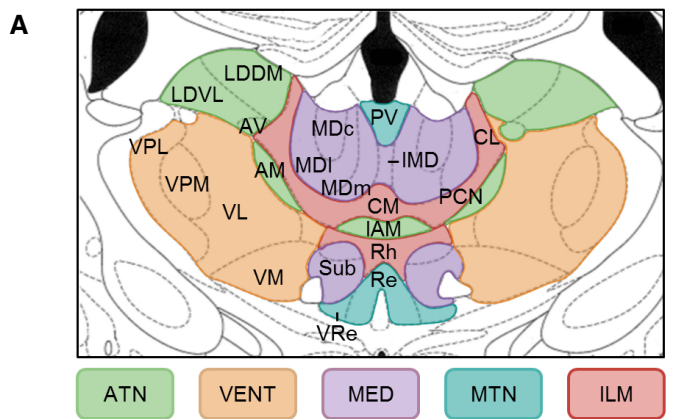


Figure EV2. ACC projections to different brain structures.

A Schematic illustration of the analyzed thalamic substructures.
 B, C Heat-maps of the z-scores based on the average ACC output density to the different thalamic (B) and hypothalamic (C) structures.

B

Region	Structure	Substructure	Z-score
TH	ATN	AM	2.07
		LD	1.34
		IAD	1.22
		IAM	0.08
		AV	-0.05
	ILM	AD	-1.1
		CL	2.06
		PF	-0.14
		RH	-0.53
		PCN	-1.17
	SPF	CM	-1.38
		SPFp	1.24
	MED	SPFm	0.77
		PR	0.65
		MD	-0.48
	VENT	SMT	-1.25
		VM	0.13
		VAL	-0.09
		SPA	-0.2
		PoT	-0.59
LAT	VPL	-1.29	
	VPL	-1.36	
MTN	LP	0.02	
	POL	-0.08	
GENv	Xi	-0.13	
	RE	-0.43	
RT	IntG	-0.24	
	IgL	-0.98	
	SubG	-1.01	
LH	LGv	-1.03	
	RT	-0.29	
		LH	-0.79



C

Region	Structure	Substructure	Z-score
Hypothalamus	MEZ	PH	0.38
		SUM	0.1
		PMd	-0.87
		PVHd	-0.93
		MM	-1.24
	LZ	ZI	1.91
		STN	-0.31
		PeF	-1.14
		PSTN	-1.18
		LHA	-1.19
		PST	-1.23

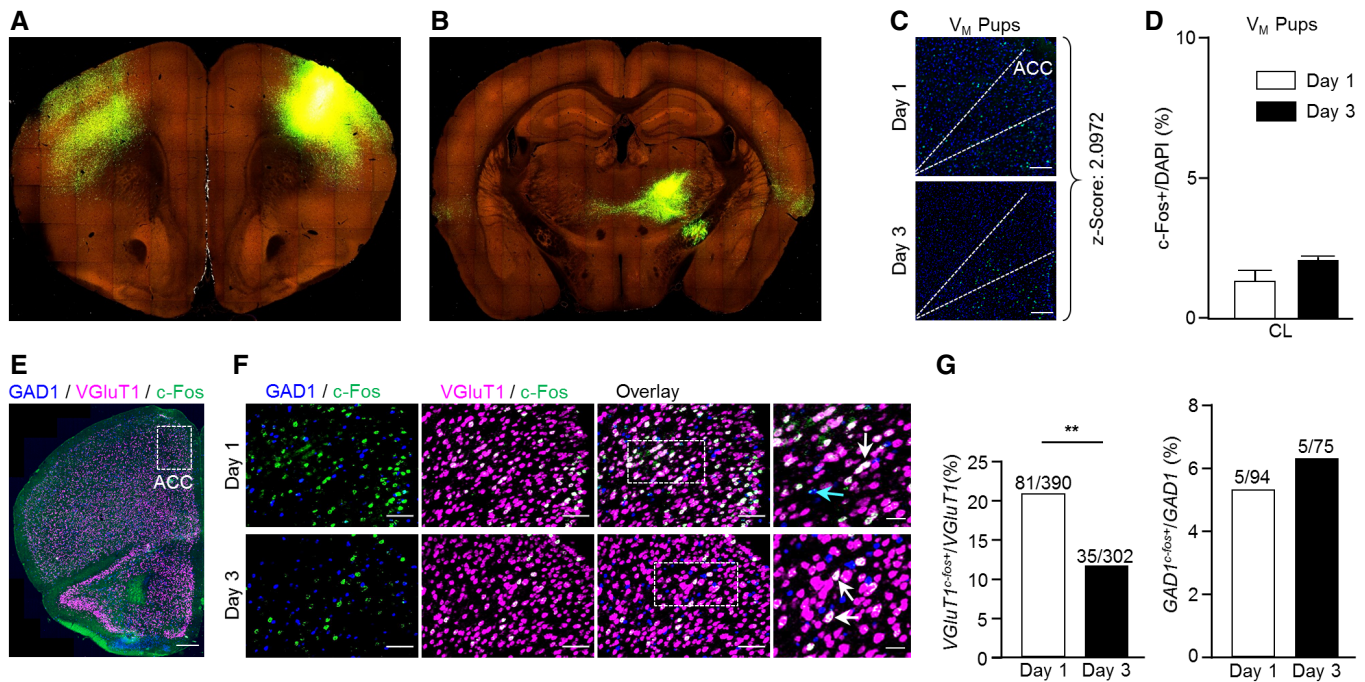


Figure EV3. Projections from the primary motor cortex to CM and PCN.

- A, B Representative coronal images from the Allen Mouse Brain Connectivity Atlas (<https://connectivity.brain-map.org/projection/experiment/100141780?imageId=102152345>), following anterograde labeling with AAV-GFP in the primary motor cortex showing site of injection (A) and axonal distribution pattern in CM and PCN (B).
- C Representative coronal sections of the mPFC immunostained for *c-Fos* with the anatomical borders of the ACC indicated by dashed lines, in virgin male mice on day 1 and 3 of behavioral testing. Scale bar 100 μ m, 20 \times magnification. Z-score for the difference in the fraction of *c-Fos*-expressing cells, between day 1 and 3 of behavioral testing.
- D Fraction of *c-Fos*⁺ cells in the CL central lateral nucleus in virgin male mice exposed to pups, on day 1 and 3 of behavioral testing (Day 1: $n = 4$, Day3: $n = 3$). Data were analyzed using Student's *t*-test and are expressed as mean \pm s.e.m.
- E, F A representative image of the PFC of a virgin female following initial pup exposure showing mRNA expression patterns of *slc17a7* (VGluT1), *gad1* (GAD1), and *c-fos* (*c-Fos*) together with high-magnification images of the ACC, following the 1st and 3rd days of pup-exposure. The scale bars 500 μ m, 10 \times magnification (A), and 100 μ m, 20 \times magnification (B).
- G A comparison of the fraction of excitatory and inhibitory *c-Fos*⁺ cells, of the total VGluT1 and GAD1 cell population in the ACC, following the 1st and 3rd pup exposure days; data were analyzed using Fisher's exact test; ** $P < 0.01$.

Source data are available online for this figure.

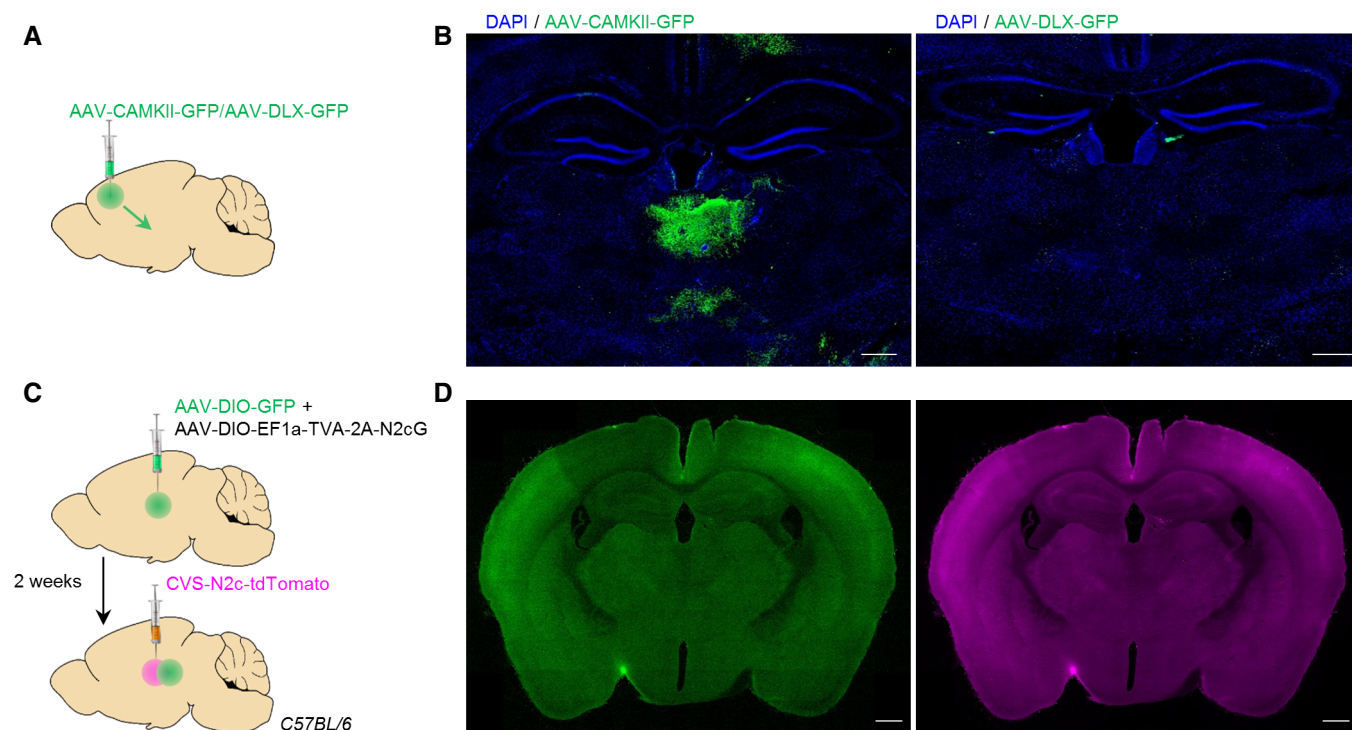


Figure EV4. Target specificity of viral vectors used for anterograde and retrograde labeling.

- A Schematic representation of the anterograde tracing from ACC excitatory/inhibitory projections to thalamic structures.
- B Representative coronal sections of the thalamic region, following the labeling schemes described in (A), for identification of axonal projections from excitatory (left) —but not inhibitory (right) ACC neurons. Scale bars 200 μm , 10 \times magnification.
- C, D Schematic representation (C) and representative coronal sections (D) of the dual anterograde and retrograde tracing to and from the CL of C57BL/6 control mice. Scale bars 500 μm , 10 \times magnification.

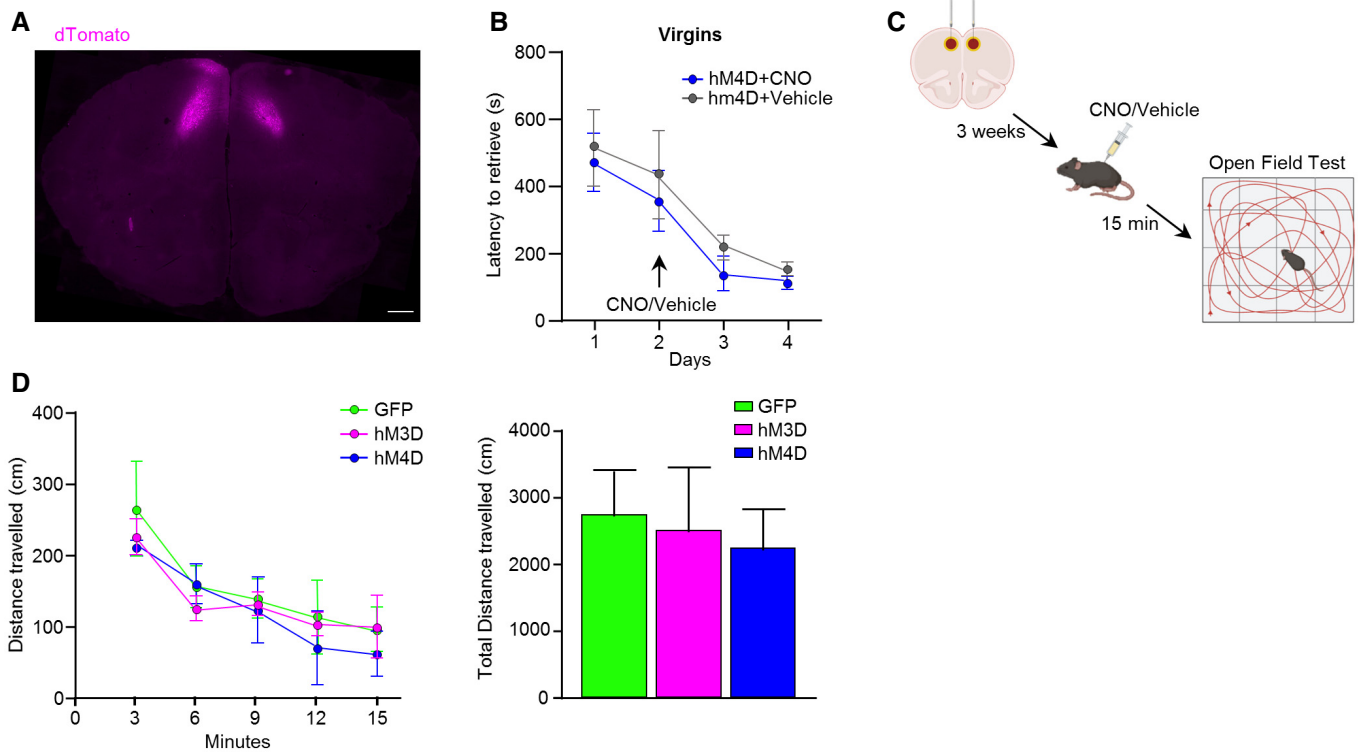


Figure EV5. Chemogenetic strategy.

A Representative coronal section demonstrating AAV-DIO-CAG-hM4D-2A-dTomato expression pattern in the ACC. Scale bar 500 μ m, 10 \times magnification.

B Quantification of pup retrieval latencies of virgin females following CNO/vehicle administration on day 2 in mice injected with hM4D into the ACC. $n = 7$ /group. Data were analyzed using two-way ANOVA with repeated measures and are expressed as mean \pm s.e.m.

C Schematic representation of the chemogenetic strategy followed by the Open Field Test. Distance traveled in virgin females injected with hM3D/hM4D/GFP after CNO administration.

D Quantification of total distance traveled of virgin females in the Open Field Test following CNO/vehicle administration in mice injected with hM3D or hM4D into the ACC ($n = 5$ /group). Data were analyzed using two-way ANOVA with repeated measures and are expressed as mean \pm s.e.m.

Source data are available online for this figure.