DREADDs Presentation

Destinee Gatlin 10/29/21

Intro

- Dr.Roth = pioneer of DREADDs (Designer Receptors Exclusively Activated by Designer Drugs)
    - Professor at UNC Chapel Hill
    - “Optogenetics is very good if you want millisecond control,” he notes. Chemogenetics, on the other hand, is easier to use, and more practical for activating larger populations of neurons. Instead of implanting light fibers all over the brain, “you can put the drug in the drinking water,” Dr. Roth explains, and simultaneously activate all the cells containing your DREADD, wherever they are located.
- Receptor designed to be activated only by designed ligands
    - GPCRs mutated to respond to synthethic ligands and not native ligands
        - CNO = very commonly used synthetic ligand

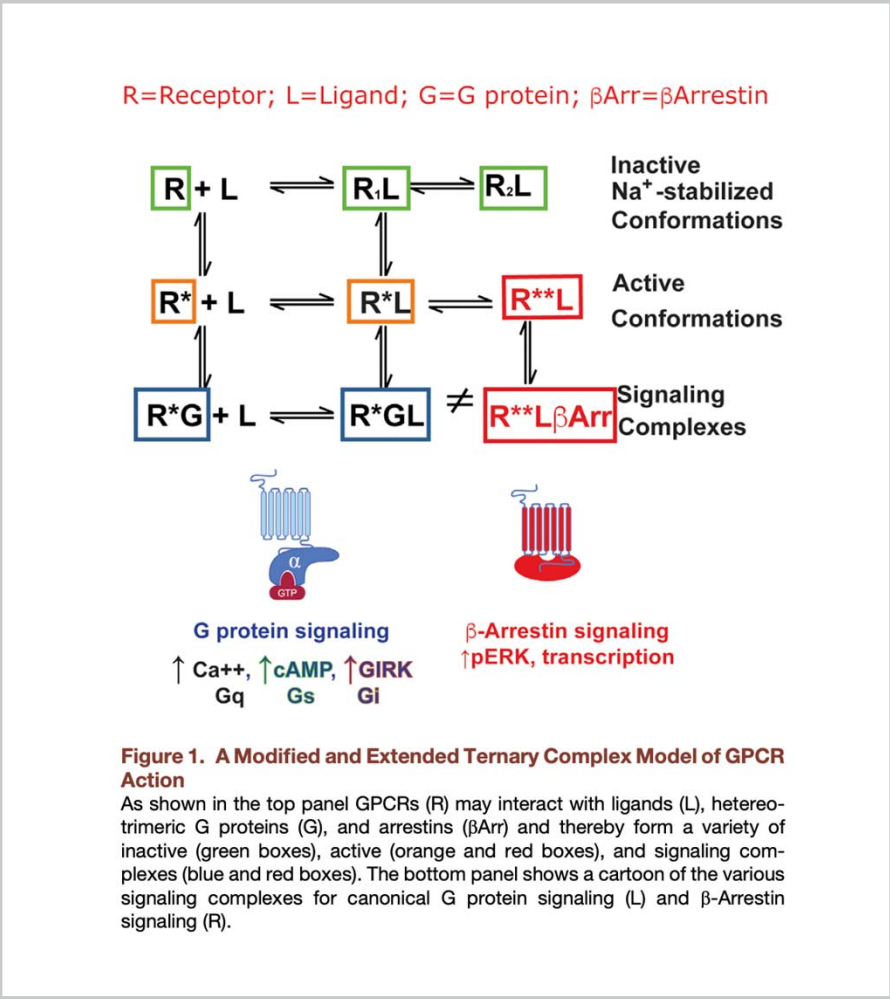
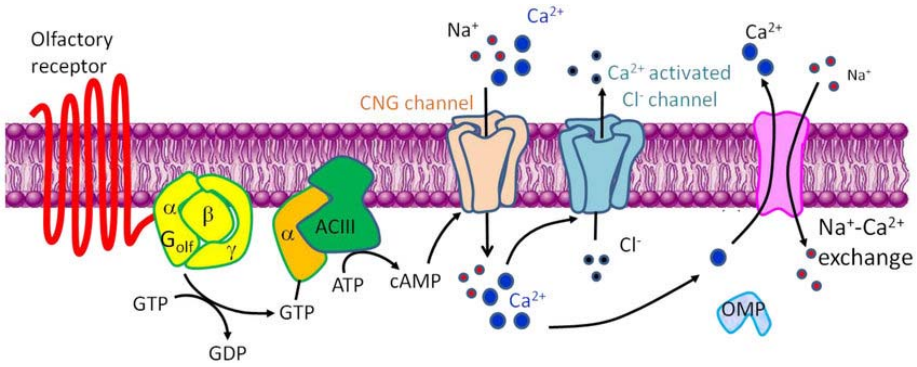
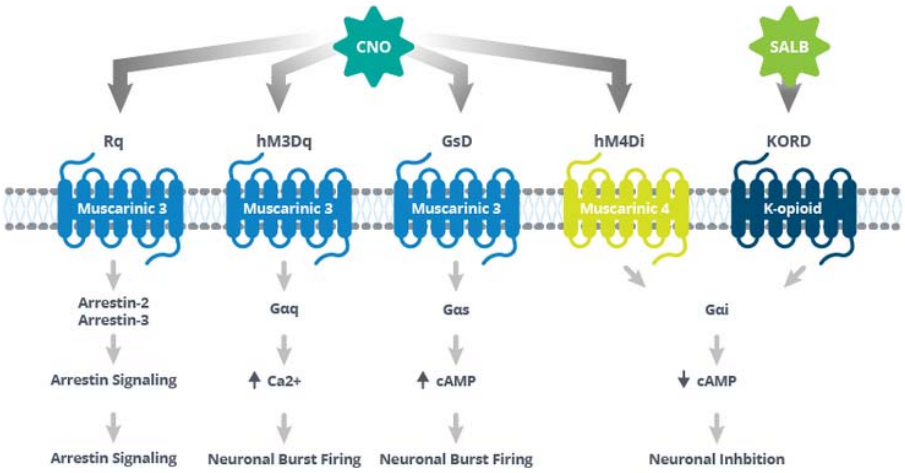


Figure 1. A Modified and Extended Ternary Complex Model of GPCR Action

As shown in the top panel GPCRs (R) may interact with ligands (L), hetereotrimeric G proteins (G), and arrestins (βArr) and thereby form a variety of inactive (green boxes), active (orange and red boxes), and signaling complexes (blue and red boxes). The bottom panel shows a cartoon of the various signaling complexes for canonical G protein signaling (L) and β-Arrestin signaling (R).





hM3Dq = initiates release of intracellular Ca2+
hM4Di = activates inward rectifier potassium channels → hyperpolarization

How do we choose a DREADD?

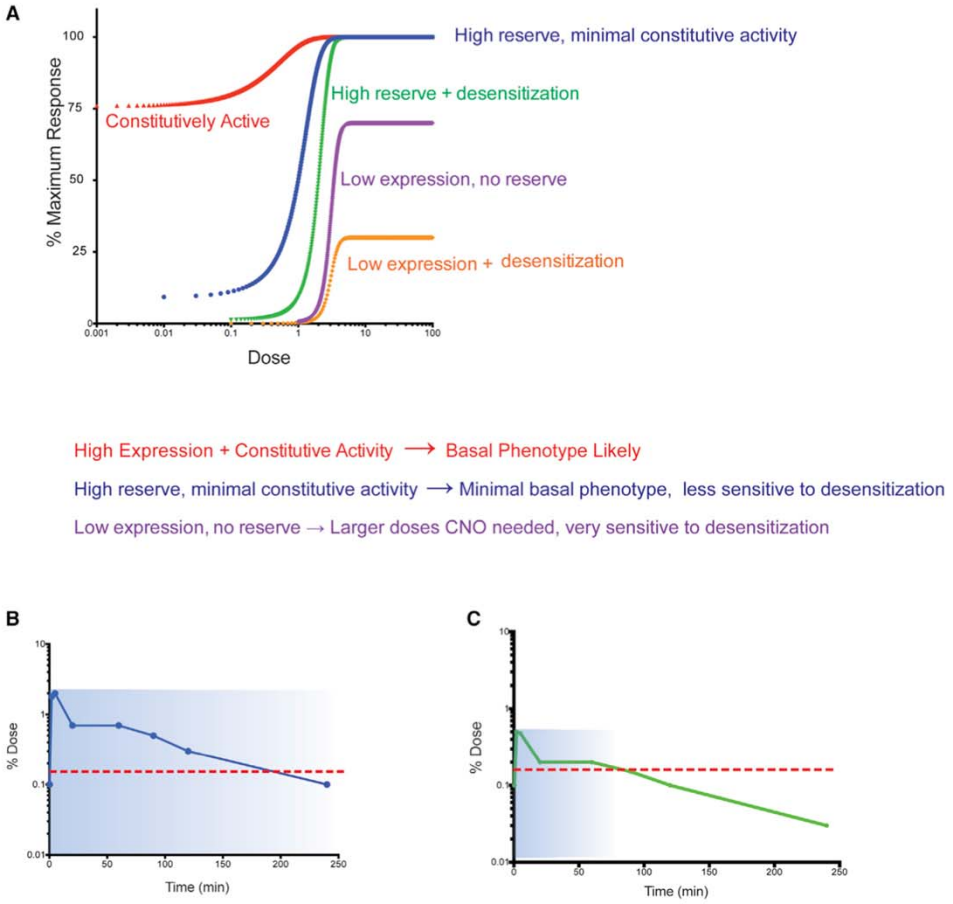


Figure 2. How Receptor Reserve and Constitutive Activity may Modify DREADD Actions In Vitro and In Vivo

(A) Simulations of receptor activity using a standard four-parameter logistic equation for GPCR activation, and variable receptor expression (DeLean et al., 1978) was used to simulate the effects of over-expression of a DREADD with constitutive activity (red circles); high receptor reserve, minimal constitutive activity (blue circles); high receptor reserve + desensitization (green circles); low expression and no receptor reserve (purple circles); and low expression, no receptor reserve, and desensitization (orange circles).

(B and C) Potential pharmacokinetic parameters of CNO following high (B) and lower (C) doses. The dotted red line indicates the threshold concentration required for activation of the DREADD in situ.

Benefits of DREADDS

- Control point of study : Use GPCRs so can be specific on region/pathway of interest
- DREADDS are non-toxic / long lasting ; repeated drug delivery
- CNO crosses the blood brain barrier → IP injections of drug; food delivery so no annoying surgical hardware required

EJN European Journal of Neuroscience

FENS Federation of European Neuroscience Societies

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BEHAVIOURAL NEUROSCIENCE

DREADD-induced silencing of the medial amygdala reduces the preference for male pheromones and the expression of lordosis in estrous female mice

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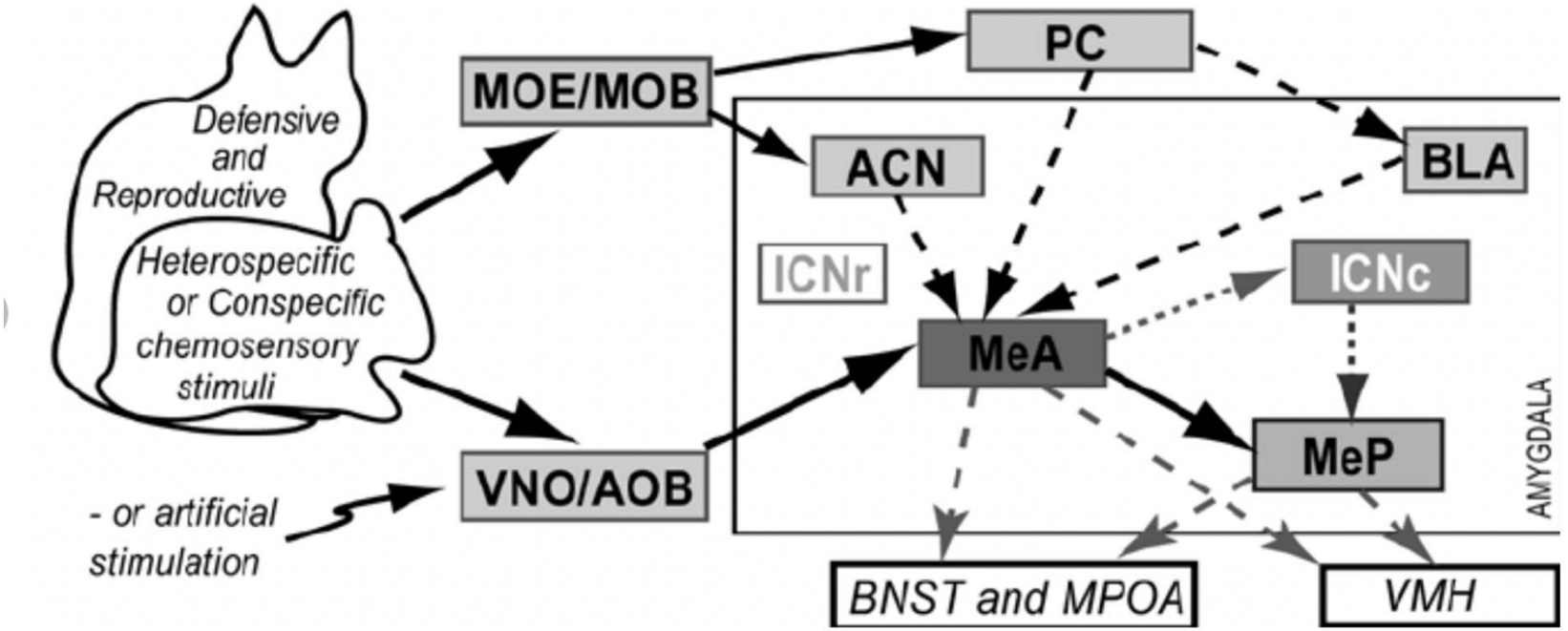
Keywords: chemogenetics, chemosensation, Fos expression, sexual behavior



Simplified diagram of chemosensory circuit in amygdala. Vomeronasal input via accessory olfactory bulb (VNO/ AOB) is analyzed in anterior and posterior medial amygdala (MeA, MeP). MeP appears to be inhibited by intercalated nucleus (ICNc) for heterospecific and artificial stimuli. MOE/ MOB: Main olfactory epithelium/Main olfactory bulb. ACN: Anterior Cortical Nucleus. PC: Piriform Cortex. BLA: Basolateral amygdala. ICNr: rostral part of medial intercalated nucleus. ICNc: caudal part of ICN. MPOA: Medial Preoptic Area. VMH: Ventro-medial hypothalamus.

Methods

- Medial amygdala (Me) = mating behavior in male and female mice
- Male and female mice: 5-7 wks old
  - 24 female mice – Me bilaterally injected with AAV + DREADDs (hM4Di) → one week later = bilateral ovariectomy → two weeks later = behavioral testing
  - 13 female control mice = no DREADDs injection, ovariectomy
- 2 days before testing: estrodial injection
- 3-6 hrs before urine collection: progesterone injection

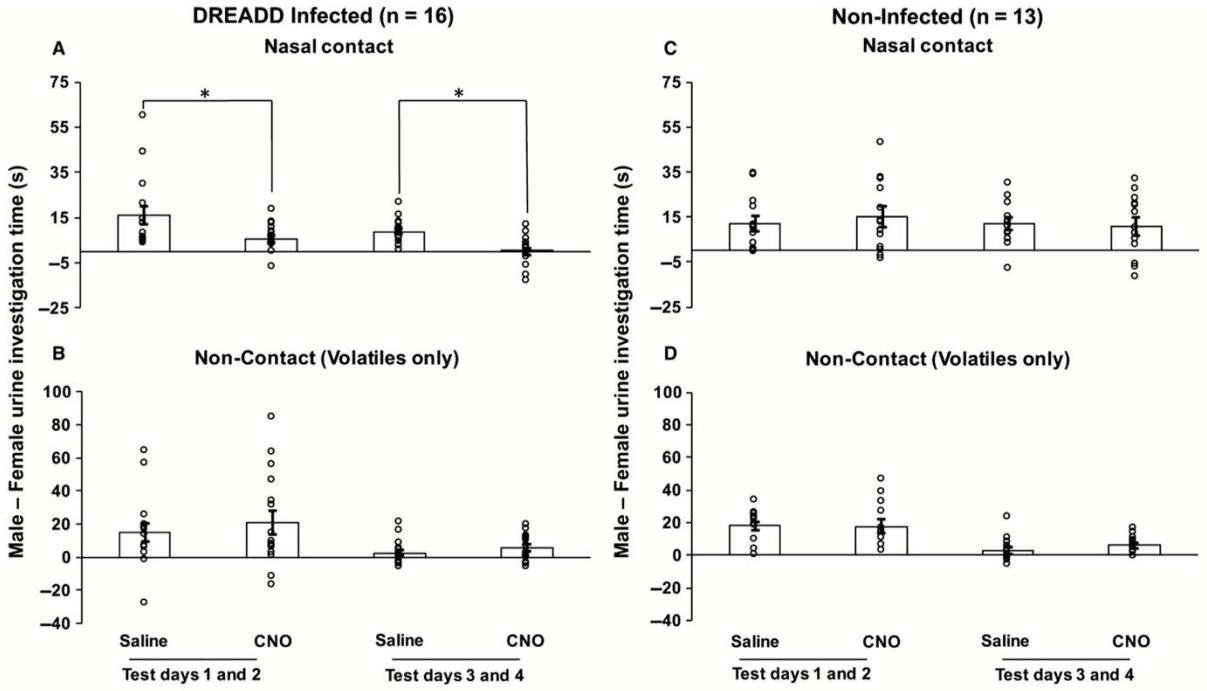
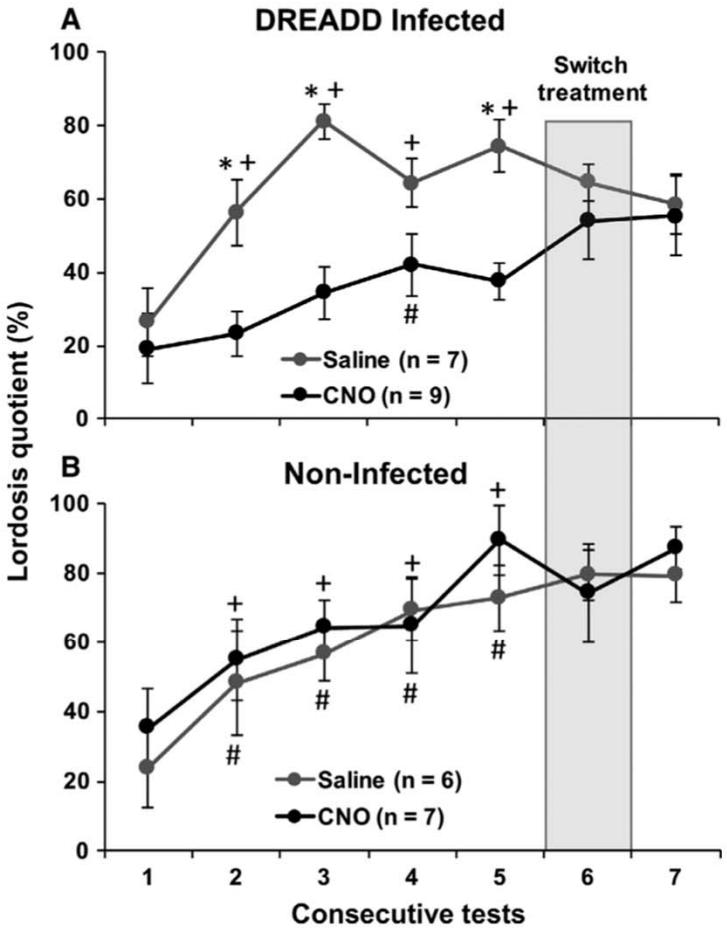
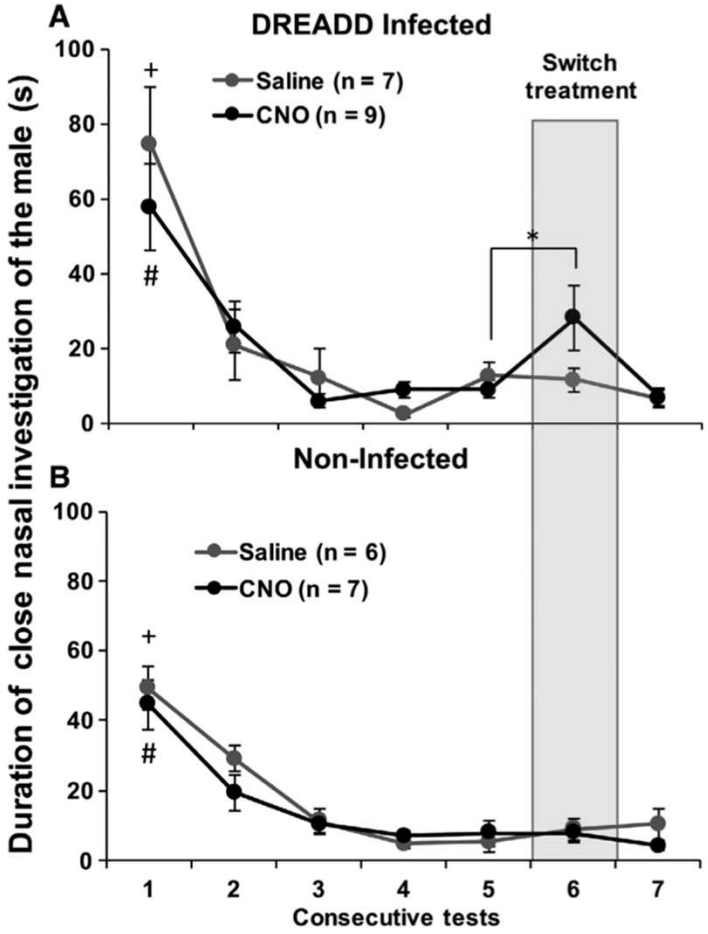


FIG. 1. The effect of clozapine-N-oxide (CNO)-induced medial amygdala silencing on the preference of ovariectomized, estradiol, and progesterone primed female mice to investigate urinary cues from testes-intact male vs. estrous female mice. Data are shown for estrous females with bilateral DREADD infections of the medial amygdala (A and B) and for non-infected subjects (C and D). Females' preference to investigate urinary chemosignals is represented as difference scores (male - female urine investigation time in seconds) in test sessions conducted on four separate days. On each test day, female subjects received two 5-min sessions: one in which they were allowed to make nasal contact with the urinary stimuli (top panels), and another in which nasal contact was prevented (non-contact, volatiles only; bottom panels). Either the DREADD activating drug, CNO, or saline was administered intraperitoneally 30 min prior to each behavioral test. The average difference score data are expressed as the mean ± SEM, while the circles represent the individual difference scores for each mouse. *$P < 0.05$ for treatment comparisons between sessions on test days 1 and 2 and between sessions on test days 3 and 4. The number of subjects in each group is shown in parentheses.


A
DREADD Infected
Switch treatment
Saline (n = 7)
CNO (n = 9)
B
Non-Infected
Saline (n = 6)
CNO (n = 7)
Lordosis quotient (%)
Consecutive tests


A
DREADD Infected
Switch treatment
Saline (n = 7)
CNO (n = 9)
B
Non-Infected
Saline (n = 6)
CNO (n = 7)
Duration of close nasal investigation of the male (s)
Consecutive tests

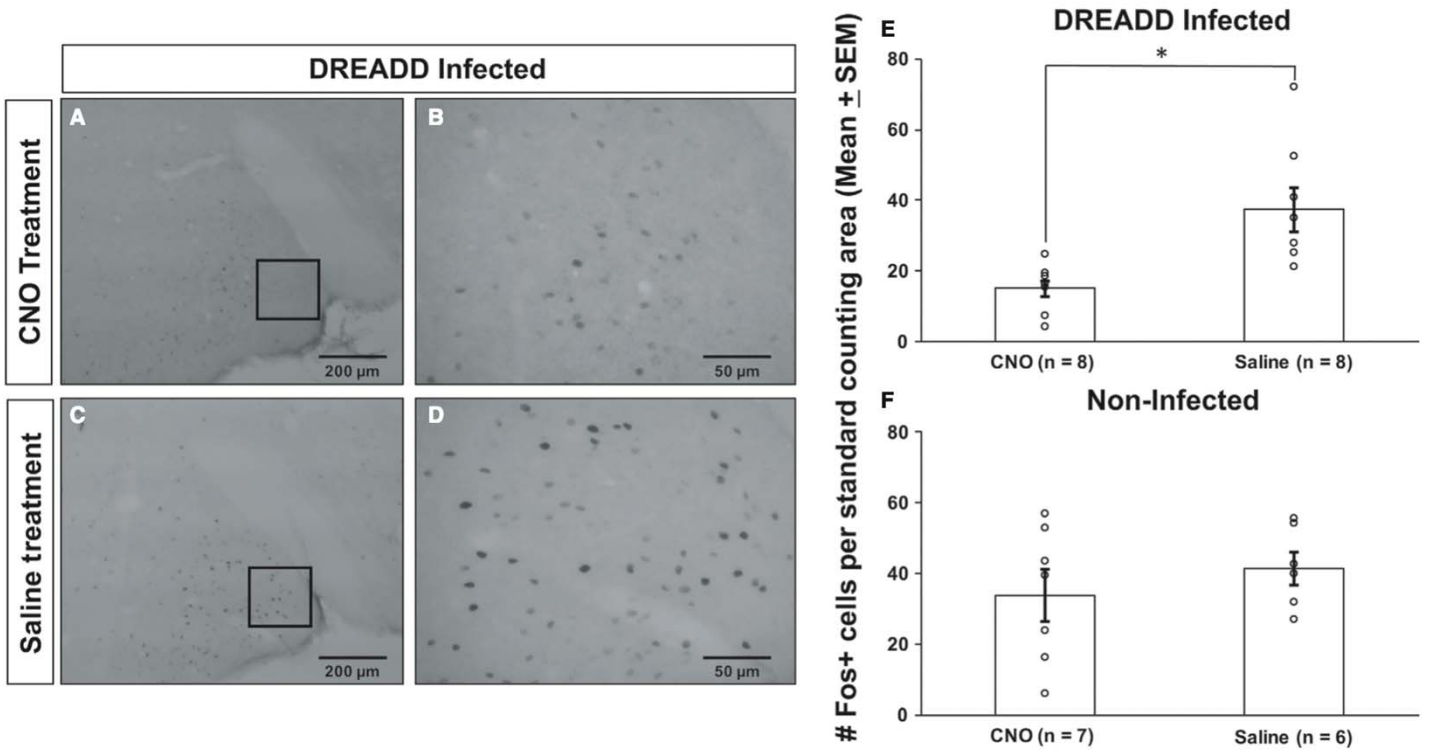


FIG. 6. Clozapine-N-oxide (CNO) injections significantly reduced the activation of medial amygdala (Me) neurons (indexed by a reduction in the number of Me Fos-IR neurons) by male pheromones in estrous female mice in cohort 1 that previously had received bilateral Me DREADD infections. Photomicrographs show representative examples of Fos staining in the Me of DREADD-infected estrous females that were placed on male bedding and treated prior to being killed with either the DREADD activating drug, CNO, (A, B), or saline (C, D). Quantification of the number of Fos-IR neurons per standard Me counting area is given for DREADD-infected (E) and non-infected (F) estrous females that were given CNO or saline injections and then exposed to male soiled bedding prior to being killed. Results are expressed as the group means ± SEM, while the circles represent the number of Fos^+ cells for each mouse. *$P < 0.05$ between treatment groups. The number of females in the four treatment groups is given in parentheses.

Sensory and Motor Systems

DREADD-Induced Silencing of the Medial Olfactory Tubercle Disrupts the Preference of Female Mice for Opposite-Sex Chemosignals

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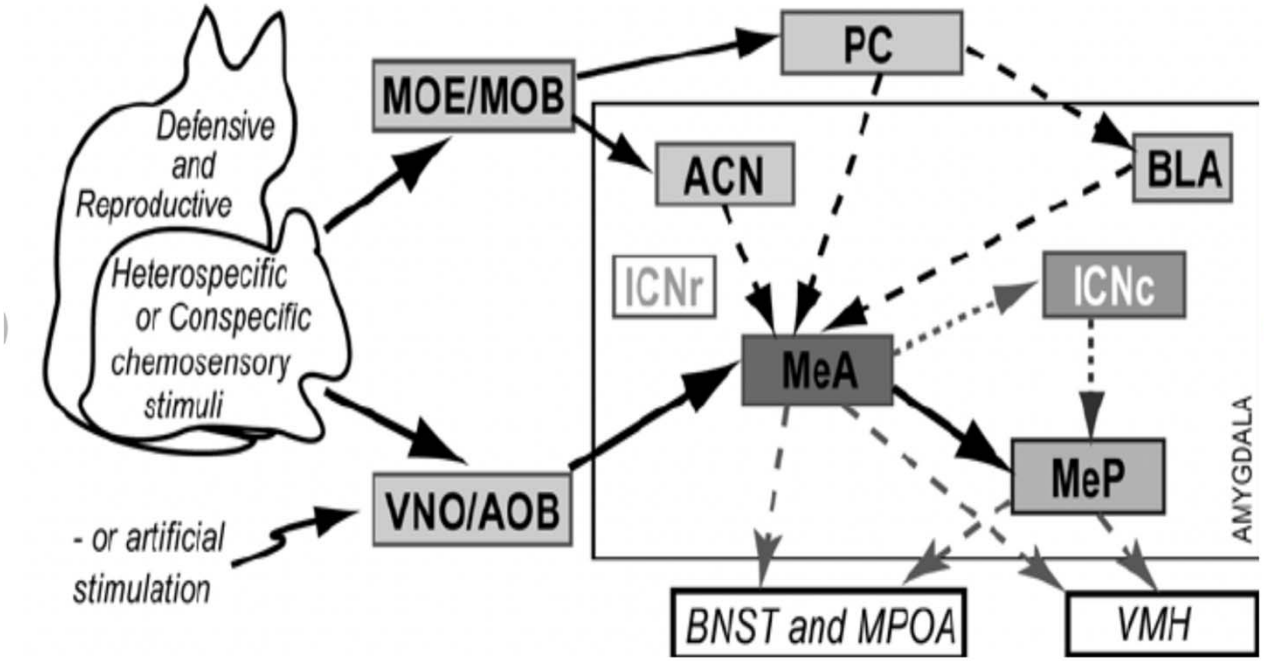

Simplified diagram of chemosensory circuit in amygdala. Vomeronasal input via accessory olfactory bulb (VNO/ AOB) is analyzed in anterior and posterior medial amygdala (MeA, MeP). MeP appears to be inhibited by intercalated nucleus (ICNc) for heterospecific and artificial stimuli. MOE/ MOB: Main olfactory epithelium/Main olfactory bulb. ACN: Anterior Cortical Nucleus. PC: Piriform Cortex. BLA: Basolateral amygdala. ICNr: rostral part of medial intercalated nucleus. ICNc: caudal part of ICN. MPOA: Medial Preoptic Area. VMH: Ventro-medial hypothalamus.

Medial olfactory tuberacle = mOT = trilaminar structure within the ventrum pallidum (basal ganglia) and receives direct input from the MOB ; found to play roles in drug abuse

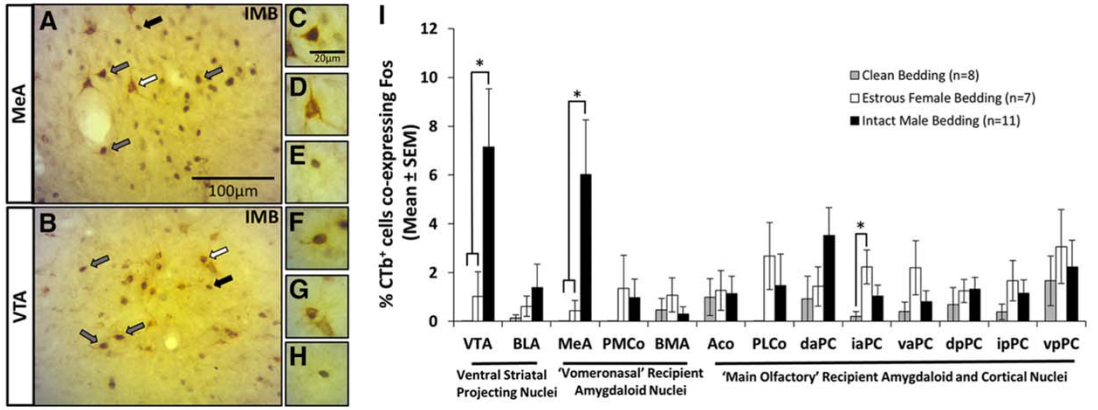

Figure 4. A subset of retrogradely labeled neurons in the MeA and VTA in female mice given a prior injection of CTb in the mOT coexpressed Fos in response to opposite-sex (male) volatile odors from soiled bedding (IMB). ***A***, ***B***, Representative photomicrographs depicting back-labeled CTb^+ (brown) and Fos^+ (black) neurons in the MeA (***A***) and the VTA (***B***). White arrows point to neurons positive for CTb; black arrows point to neurons positive for Fos; gray arrows point to neurons positive for both CTb and Fos (CTb^+/Fos^+). ***C–H***, High-magnification (100×) photomicrographs depicting colabeled (CTb^+/Fos^+) neurons identified by gray arrows in the MeA (***C***) and VTA (***F***), neurons positive for CTb only identified by white arrows in the MeA (***D***) and VTA (***G***), and neurons positive for Fos only identified by black arrows in the MeA (***E***) and VTA (***H***). ***I***, Effect of volatiles emitted from soiled bedding on the expression of Fos in various forebrain neurons of estrous female mice that were retrogradely labeled by a prior injection of CTb into the mOT. The mean percentage (±SEM) of CTb-labeled (mOT-projecting) cells that coexpressed Fos in response to volatiles from clean bedding, estrous female soiled bedding, or testes-intact male soiled bedding is shown in 13 forebrain regions where Fos/CTb colocalization was observed. $*p \leq 0.05$ (SNK *post hoc* tests following a significant overall ANOVA). In the legend, *n* refers to the number of subject mice in each group. See the legend of Figure 3 for definitions of brain region acronyms.

Male urine soiled bedding evokes MeA activity but not female urine soiled

- 5-6wk old mice
  - Ovariectomy → week recovery → DREADD delivery (hM4Di) into medial olfactory tuberacle (mOT) → subQ estradiol (E2) implantation
- Martel and Baum found that removal of the MOE diminishes opposite sex odor interest

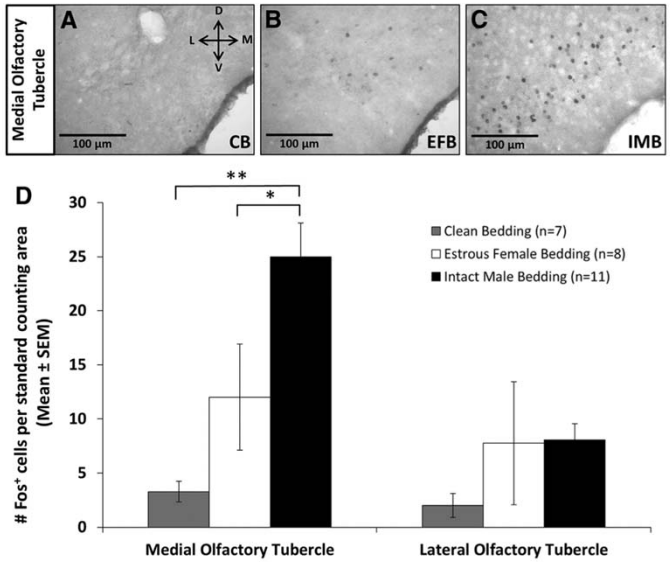

Figure 2. mOT neurons, but not lOT neurons, were selectively activated in female mice by volatiles emitted from opposite-sex (male) soiled bedding. ***A–C***, Representative photomicrographs depicting Fos protein immunoreactivity in the mOT of female subject mice exposed to volatiles from clean bedding (CB; ***A***), estrous female soiled bedding (EFB; ***B***), and testes-intact male soiled bedding (IMB; ***C***). ***D***, Average number of Fos-IR cells (±SEM) observed in the mOT and lOT in response to volatiles from clean bedding, estrous female soiled bedding, or testes-intact male soiled bedding. $*p < 0.01$; $**p < 0.001$ (SNK *post hoc* tests following a significant overall ANOVA). In the legend, *n* refers to the number of subject mice in each group.

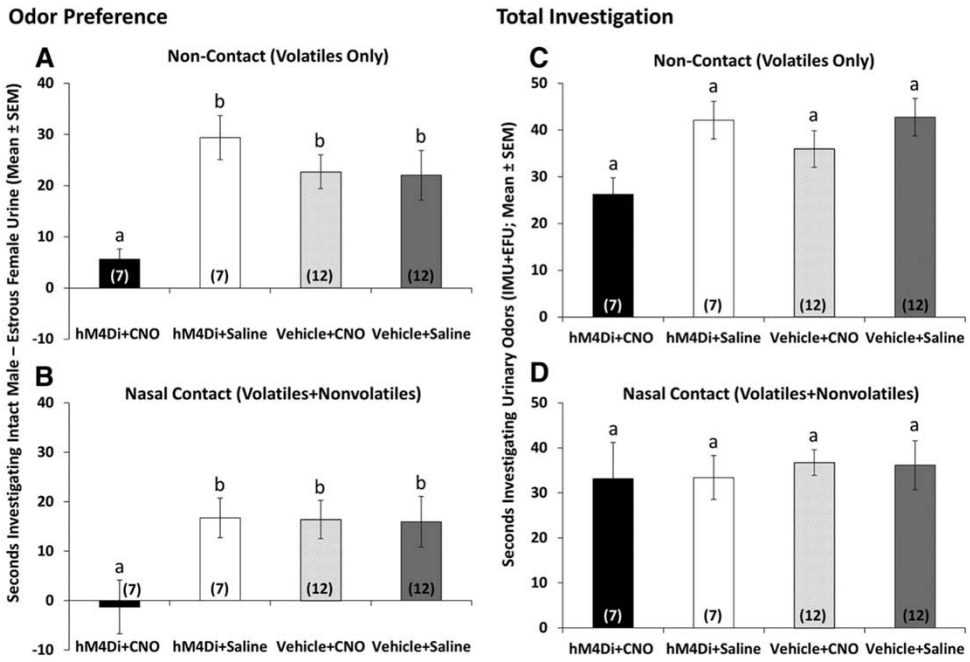

Figure 5. ***A***, ***B***, Effect of bilateral CNO-induced mOT silencing ($hM_4Di+CNO$) on the preference of ovariectomized, estradiol- and progesterone-primed female mice to investigate urinary odors from estrous female vs testes-intact male mice presented simultaneously in the home cages of subject mice. ***A***, ***B***, The preference of subject mice for volatile urinary odors presented outside the home cage (Non-Contact – Volatiles Only; ***A***) and the preference of subject mice for volatile plus nonvolatile urinary odors presented inside the home cage (Nasal Contact − Volatiles+Nonvolatiles; ***B***). Data are represented as the average (±SEM) time spent investigating intact male urine minus the time spent investigating estrous female urine for each group. Different letters above the columns in each group indicate statistically significant differences from each other (two-way repeated-measures ANOVA with one-factor repetition followed by SNK *post hoc* test). The number of subject mice in each group is given within columns in parentheses. ***C***, ***D***, Effect of bilateral CNO-induced mOT silencing ($hM_4Di+CNO$) on the total amount of time ovariectomized, estradiol-primed, and progesterone-primed female mice spent investigating urinary stimuli. ***C***, Total amount of time subject mice spent investigating intact male plus estrous female urinary volatiles (Non-Contact − Volatiles Only). ***D***, Total amount of time subject mice spent investigating intact male plus estrous female urinary volatiles and non-volatiles (Nasal Contact − Volatiles plus Nonvolatiles). Data are represented as the average (±SEM) time spent investigating intact male urine plus the average time spent investigating estrous female urine for each

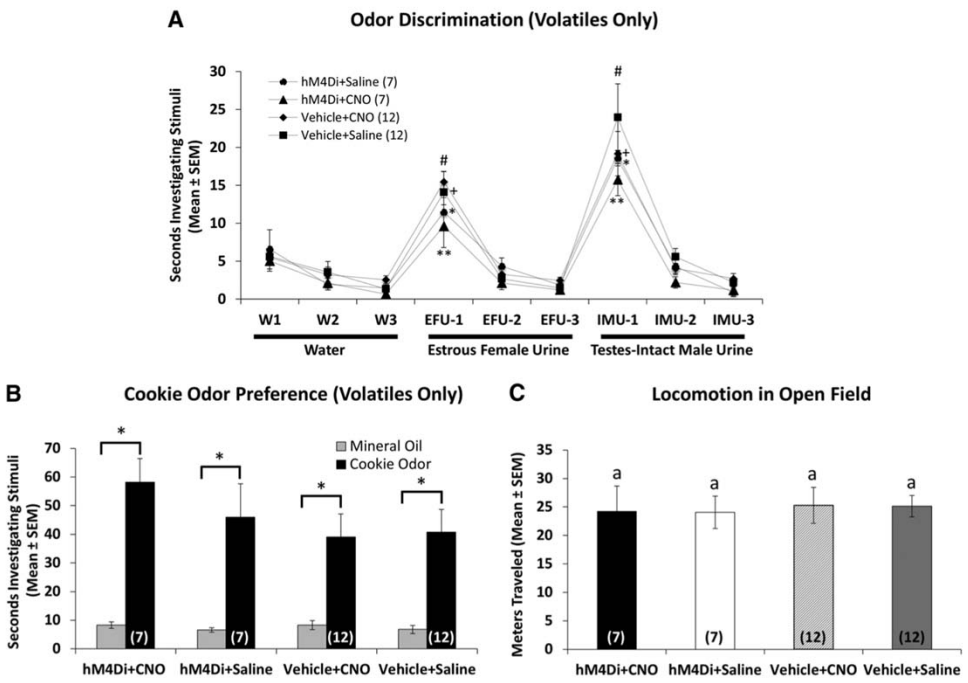

Figure 6. ***A,*** Effect of bilateral CNO-induced mOT silencing ($hM_4Di+CNO$) on the ability of ovariectomized, estradiol-primed female mice to discriminate between testes-intact male and estrous female volatile urinary odors presented outside of the home cage (volatiles only). Each stimulus was presented three consecutive times. Estrous female versus testes-intact male urinary volatiles were reliably discriminated by all groups (paired *t* test comparisons of mean investigation times of third water versus first female urine, and third female urine versus first male urine; $*p < 0.001$; $+p < 0.001$; $\#p < 0.001$; $**p < 0.001$). No between-group differences in the level of investigation during the first presentation of estrous female urine and the first presentation of intact male urine were observed ($p > 0.05$, two-way repeated-measures ANOVA with one-factor repetition). ***B***, Effect of bilateral CNO-induced mOT silencing ($hM_4Di+CNO$) on the preference of ovariectomized, estradiol-primed female mice to investigate volatiles emanating from cookie odor dissolved in mineral oil vs mineral oil alone presented simultaneously in the home cages of subject mice. Data are represented as the mean (±SEM) time spent investigating each odor ($*p < 0.01$, paired *t* test comparisons of mean investigation times for each odor). ***C***, Effect of bilateral CNO-induced mOT silencing ($hM_4Di+CNO$) on locomotion displayed by ovariectomized, estradiol-primed female mice. Data are represented as the mean (±SEM) distance traveled (in meters) in a 20 min open field test ($p > 0.05$, two-way repeated-measures ANOVA with one-factor repetition). The number of subject mice in each group is given within columns in parentheses.

Current Biology
Article

Encoding of Odor Fear Memories in the Mouse Olfactory Cortex

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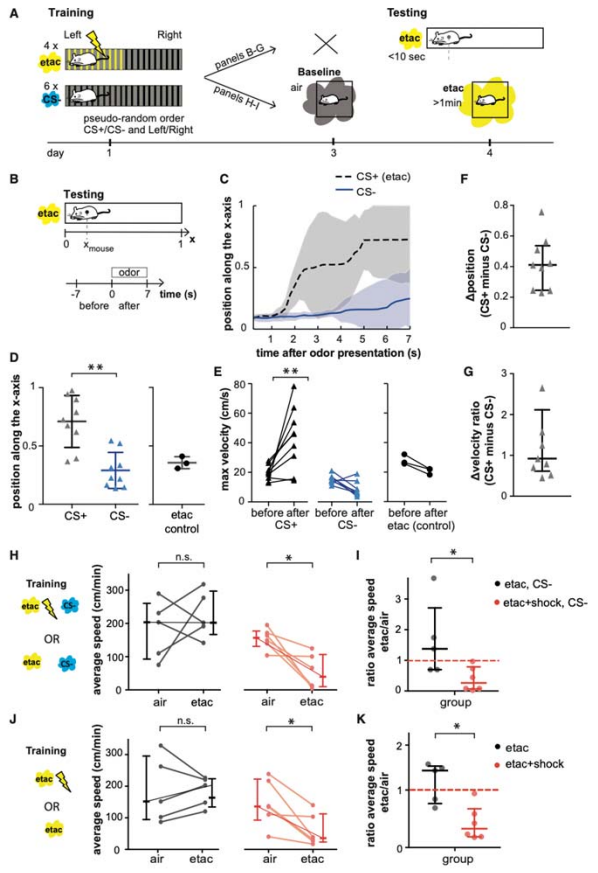
Methods

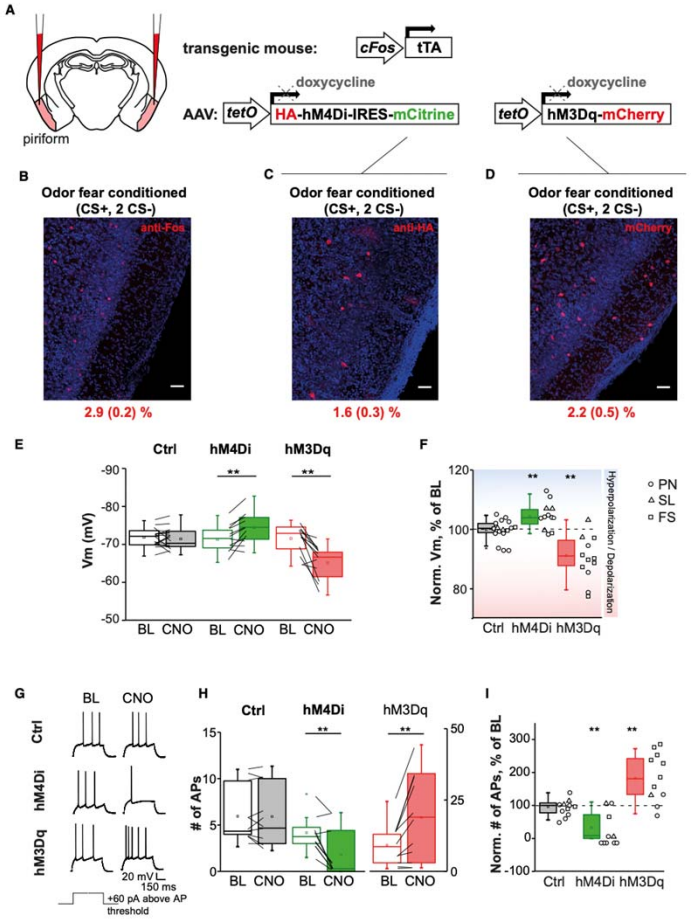
Piriform cortex = largest cortical area that receives input from the MOB; olfactory learning

Classical conditioning: conditioned reinforced stimulus = ethyl acetate + foot shock; escape foot shock by running to opposite side of the box

- Injected Hm4Di DREADD (via AAV) into the piriform cortex in *cFos*-tTA transgenic mice



Recall: hM3Dq increases intracellular Ca2+



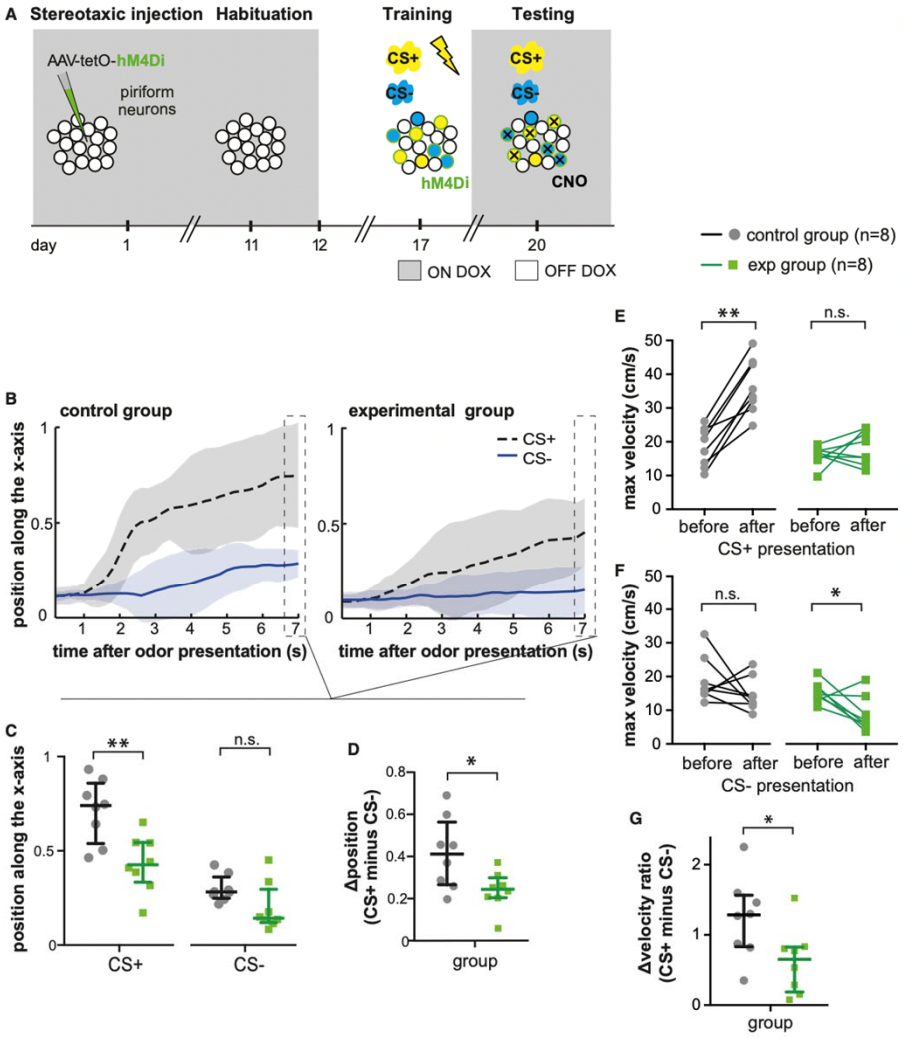


Figure 3. Silencing Fos-Tagged Piriform Ensembles Impairs Odor Fear Memory Recall

(A) Experimental design: *cFos*-tTA transgenic mice (experimental group, n = 8) or wild-type mice (control group, n = 8) are injected with AAV-tetO-hM4Di in both hemispheres of the PCx. Ten days later, mice are habituated to the training and testing boxes. Upon doxycycline (DOX) removal, mice are fear conditioned, and hM4Di expression (green circles) is induced in Fos-tagged neurons (filled in blue and yellow; see Figure S4). Mice are returned to a DOX-containing diet to avoid further expression of hM4Di. Three days later, all mice are intraperitoneally injected with CNO, which silences hM4Di-expressing neurons (black crosses), and memory retrieval is tested in the rectangular testing box (Figure 1B).

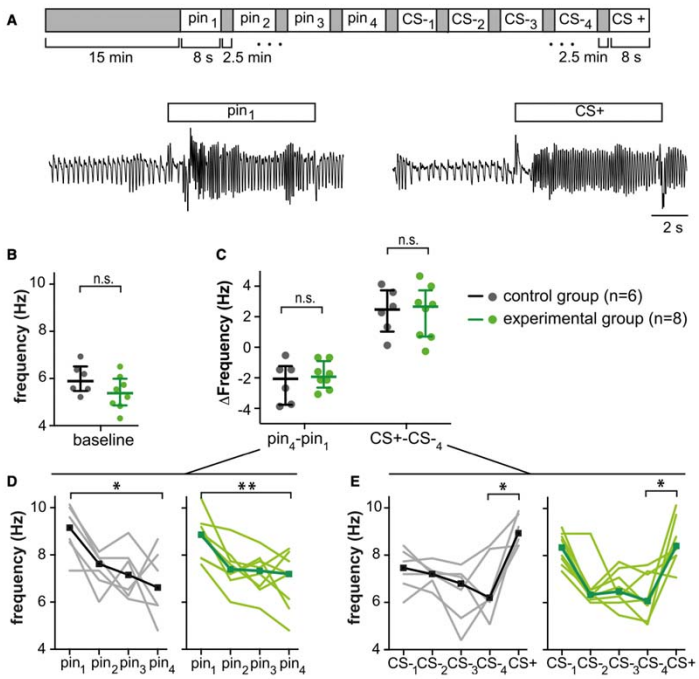


Figure 5. Silencing Fos-Tagged Piriform Ensembles Does Not Interfere with Odor Detection and Discrimination

(A) Top: experimental design: Fos tagging during olfactory fear conditioning is performed as described in Figure 3 (see also Figure S4). Three days after fear conditioning, sniffing behavior is quantified in a plethysmograph. Mice are intraperitoneally injected with CNO and habituated to the plethysmograph for 15 min, and each odor is then presented for 8 s with a 2.5 min inter-trial interval. Pin, pinene; CS^{-}, beta-citronellol; CS^{+}, ethyl acetate. Bottom: representative sniff recordings from one mouse when exposed to pinene (1st presentation) or the CS^{+}.

(B and C) Dots represent individual mice, and averaged data are shown as median and interquartile range.

(D and E) Each thin line represents data of individual mice; the squares and thick lines represent averaged data (median).

(B) Baseline sniff frequency, corresponding to the averaged sniff frequency 8 s before odor onset.

(C–E) For both the control (n = 6) and experimental groups (n = 8), repeated exposure to the same odor resulted in a decrease in the sniff frequency (habituation), whereas presentation of a different odor resulted in an increase in the sniff frequency.

(C) The changes in sniff frequency after presentation of the same or a different odor are similar between the experimental and control groups. The sniff frequency during the fourth presentation of pinene is subtracted from its first presentation (pin4 − pin1), or the sniff frequency during the fourth presentation of the CS^{-} is subtracted from the sniff frequency during the CS^{+} presentation ($CS^{+} - CS^{-}4$).

(D) Sniff frequency for the first to fourth presentation of pinene: control (left) and experimental group (right).

(E) Sniff frequency for the first to fourth presentation of the CS^{-}, and subsequent presentation of the CS^{+}: control (left) and experimental group (right).

*p < 0.05, **p < 0.01; n.s., not significant.

Conclusions

- DREADDs allow refined and direct targeting of regions of interest
  - Controllable
  - Long lasting
  - Less cumbersome
  - Broad use
- Future of DREADDs: to have activating and inactivating DREADDs act on same neuron