UNIVERSITY OF CALIFORNIA SAN DIEGO

Functional, Spatial Organization of Posterolateral Cortical Amygdala in the Control of Odor Evoked Behavioral Valence

A thesis submitted in partial satisfaction of the requirements for the degree of Master of Science

in

Biology

by

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The Thesis of James Hyeokjun Lee is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Table of Contents

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ABSTRACT OF THE THESIS

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Master of Science in Biology

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Professor Cory M. Root, Chair

Posterolateral cortical amygdala (PLCoA) is known to participate in innate behavior response towards odor stimuli. The participation of PLCoA is not restricted to one end of the spectrum: they take part in both aversive and appetitive behaviors that are triggered by exposures to a variety of different odors. By selectively targeting subregions of PLCoA along the anterior-posterior axis and activating the subregions through optogenetic methods, we were able to demonstrate that the posterior subregions incite attraction when activated, while the anterior subregion triggers aversion. Further tests using *Arc*-CreER^{T2} mice, which allowed the selective activation of the aversive fox odor, 2,3,5-Trimethyl-3-thiazoline (TMT), the fox odor that is innately

aversive to rodents, indicated that the TMT responsive neurons of the posterior PLCoA is, in fact, not sufficient by themselves to cause aversive behavior, unlike its anterior counterpart, which was sufficient for aversion. These data suggest the existence of spatial organization of neurons across the anterior posterior axis within PLCoA tied strongly to the innate behavioral response towards the odor stimulation.

Introduction

The sense of smell plays an important role in survival of animals, enabling them to react to signals of resources, dangers or social interactions. This is possible even without prior experience due to the existence of genetically determined neural circuits that have developed through evolutionary advantages over generations. Such behaviors can be analyzed by measuring the approach-avoidance responses towards the odor source [1, 2].

The olfactory system recognizes odors through the receptor cells that make up the nasal epithelium. The population of epithelium neurons recognizes specific odorants through a broad selection of cell-specific receptor expression [3, 4]. The mouse genome encodes approximately 1500 odorant receptors genes, providing the ability to detect a massive number of odorant molecules [3,4,5]. Sensory neurons expressing the same receptor converge their axons on to olfactory glomeruli in the olfactory bulb forming a map of olfactory sensory neuron type [6,7,8]. Within the olfactory bulb, the odor signal is coarsely mapped by functions: glomeruli within a subregion of the dorsal olfactory bulb mediate aversive responses, whereas the ventral olfactory bulb plays a role in social interaction [9,10].

The activity of individual glomeruli is carried by the second order mitral and tufted cells to multiple third order olfactory structures including entorhinal cortex, piriform cortex, olfactory tubercle, accessory olfactory nucleus, and posterolateral cortical amygdala (plCoA) [11, 12]. Previous work found that the projections from individual olfactory glomeruli to plCoA are stereotypically mapped, with each olfactory glomerulus projecting to a unique location in plCoA thus maintaining the spatial organization [11]. In

contrast, the olfactory bulb to piriform cortex projections diffuse across the piriform cortex without maintaining any spatial organization [11]. The olfactory bulb-plCoA projection is necessary for the innate aversion and attraction to specific odor cues. In addition, plCoA neurons are able to drive odor-like behavior upon exogenous stimulation. Therefore, plCoA plays a critical role in mediating the innate approach-avoidance behaviors [1].

Previous work found that different odors activate varying distributions of neurons across the anterior-posterior axis of plCoA. Neurons responsive to aversive odors were distributed evenly across the whole plCoA, while appetitive odors mostly activate neurons in the posterior region of the plCoA [1]. For example, the aversive fox odor, 2,3,5-Trimethyl-3-thiazoline (TMT), activates neurons across the entire plCoA, whereas the attractive rose odor, 2-phenyethanol (2PE) only activates neurons in the most posterior region [1]. The observation that the aversive odor activates the anterior domain while the appetitive odor does not suggest that the anterior region could be dedicated for the generation of innate aversion. On the other hand, activation of the posterior region of the plCoA by both aversive and appetitive odor suggests that the region may be heterogeneous but should contain neurons that drive appetitive responses. We have investigated this possibility by exogenous activation of neurons along the anterior-posterior axis while assaying behavioral response. First. We activated random ensembles of neurons, second, we activated odor-responsive neurons showing this gradient, and third, we attempted to silence anterior and posterior regions to look at odor responses.

Methods

Animals

The animals used for the random activation experiment were c57Bl/6j mice purchased from Jackson Laboratory. The *Arc*-CreER^{T2} mice were previously described [1] and bred in house. All of the mice were male and 8~12 weeks old at the start of the experiment. Mice were housed in a 12-12 hour inverse light cycle, and the behavioral experiments were performed during the dark phase. The surgical procedures were performed when the animals are 8 to 12 weeks old.

Stereotactic surgery

Animals were anesthetized with isoflurane infused oxygen (1-2%, 1L/min) and placed in a stereotaxic system. For analgesic drug, buprenorphine was administered. The skin on the head was clipped, then cleaned with betadine solution and 70% ethanol solution. An incision was made to expose the skull and a single craniotomy was made above the target structure using a microdrill. The injection coordinates relative to bregma used for anterior plCoA were: -1.3 anterior-posterior, -2.8 medial-lateral, -5.95 dorsal-ventral, and for posterior were: plCoA at -1.9 anterior-posterior, -3.0 medial-lateral, 5.9 dorsal-ventral. Due to variation in targeting along the anterior-posterior axis, it resulted in targeting throughout the AP axis; post hoc analysis allowed us to plot behavior in relation to its anterior-posterior coordinate. Channelrhodopsin virus (AAV5-Syn-Chr2-mCherry) was injected at the coordinates using needles pulled from capillary glass (Drummond) using automated pressure injection with a nanoject (Drummond), injecting a total volume of 150 nl at 5 nl/sec. For experiments using *Arc*-CreER^{T2} mice, Cre-dependent virus (AAV5-DIO-ChR2-eYFP) was used.

For all mice, the fiber optic embedded in ceramic ferrule was implanted at a coordinate slightly dorsal to the viral injection at -5.7 dorsal-ventral 15 minutes after the viral injection. With the fiber optic in place, primer, adhesive, and light curing dental cement (Tetric EvoFlow E3) was applied to the skull, then exposed to blue light for curing. The dental cement was then coated with a layer of black dental acrylic (Ortho Jet). For halorhodopsin expression, the same procedure was followed, but bilaterally. The virus injected was:(AAV5-Syn-eNpHR3.0-eyFP).

During the surgical procedure, the heart rate, oxygen level, body temperature, and toe pinch response was recorded every 15 minutes, as well as the body weight before and after the procedure, analgesic treatment time, and anesthesia treatment time were recorded.

Postoperative Care

After surgery, the animals were housed individually in cages with extra head room. Animals were given buprenorphine injection every 12 hours 6 times after the surgery or a single dose of sustained release buprenorphine at the start of surgery, and the weights were recorded every day for 5 days after surgery. Animals were monitored daily for health and well-being, and their weights were recorded twice per week, until they were euthanized at the end of the experiments.

For experiments using *Arc*-CreER^{T2} mice, the animals were administered tamoxifen solution (tamoxifen 2 mg/ml, ethanol 10%, corn oil 90%) by intraperitoneal injection one week after the viral injection and fiber implantation, then exposed to a cotton tip with 4 µL of TMT odor 6 hours after tamoxifen injection. This process was done in an enclosed environment with minimal outside light, sound, or odor.

Behavior

3 weeks after the surgical procedure, animals were tested in the 4-quadrant behavior chamber, an open chamber with air flow into each of the four corners and a vacuum in the middle [1]. The fiber optic implants on the animals were connected to a laser with wavelength of 473 nm for channelrhodopsin and 561 nm for halorhodopsin with custom made patch cords with ceramic sleeves that fit to the ferrule on animal's head, with single or beam splitting rotary joints in between the laser and the test subject. Each trial of the experiment lasted 25 minutes, with the first 10 minutes serving as the baseline, without any odor or laser stimulation, to test for any inherent spatial bias. If there was any bias defined as spending 25% above or below chance levels in any quadrant without stimulation, the trial would be immediately terminated and the animal would be retested on another day, to get animals acclimated to the tether and the chamber so that they would run without bias. In the latter 15 minutes, the set stimulations, odors and/or laser pulses, were released as the animal entered the designated stimulation quadrant by custom software written in Labview. first 2 minutes and last 3 minutes of the 15 minutes period were excluded from analysis as previously done.

For channelrhodopsin experiments, when the animal entered the lower left quadrant during the latter 15 minutes of the test trial, the laser pulsed at varying frequency (from 1-10 Hz) depending on the position of the animal relative to the corner of the stimulation quadrant: closer the animal is to the quadrant corner, higher the laser pulse frequency. Each animal was tested twice for the full 25-minute trials, each trial at least a day apart from the other, and the results were averaged.

For halorhodopsin experiments, the animals were tested four full trials each, TMT without any laser stimulation, TMT with 561 nm laser with 10~12 mw power at each fiber, 2-Phenylethanol without any laser stimulation, and 2-Phenylethanol with 561 nm laser. For these trials the laser was constantly on during the entirety of latter 15 minutes of each trial as previously done [1].

Histology

After the behavioral trials, the animals were assayed for light activation using c-Fos as a marker of active neurons. The Channelrhodopsin injected mice were tethered to the patch cord connected to 473 nm blue laser, which pulsed at 10Hz with average power of 7 mW for 5 minutes. The halorhodopsin injected mice were tethered bilaterally with two patch cords connected to 561 nm yellow laser, with each patch cord having non-pulsating yellow laser output of 10 mW average, with a cotton swab with TMT exposure at the same time. The odor stimulation exposure occurred for 5 minutes and the laser for 15 minutes.

An hour after the stimulation, the animals were injected with ketamine and xylazine solution and euthanized by transcardiac perfusion with 10 mL of cold PBS, followed by 4% paraformaldehyde PBS solution. The brain was extracted, placed in 4% paraformaldehyde PBS solution for at least a day, then sectioned coronally on a vibratome (Leica VT1000). The sections were treated with antibodies (titer of 1:1000): primary antibodies rabbit-anti-c-Fos (cell signaling tech) and goat-anti-GFP (Abcam); secondary antibodies: Alexa 488 anti-rabbit, Alexa 561 anti-rabbit, Alexa 488 anti-goat.

The sections were mounted on Superfrost plus slides (Fisher) and stained with mounting medium with DAPI (southern biotech), then scanned with the Olympus VS120

slide scanner. Scanned slides were analyzed with ImageJ using particle analysis function.

Results

We hypothesized that plCoA is spatially organized with an axis of valence across the anterior-posterior axis. If the hypothesis is true, it can be predicted that exogenous activation of neurons at various points along the axis should elicit correlated degrees of avoidance and approach responses. To test this, we asked whether exogenously activating random sets of neurons in small regions across the anterior and posterior axis of plCoA in naive mice was sufficient to produce either aversive or appetitive response towards the stimulation. An adeno-associated virus encoding channelrhodopsin-mCherry was injected to the targeted plCoA area unilaterally, and a fiber optic was implanted above the injection site. The subjects were introduced to a 4 quadrant open field assay and were tested for response to activation of neurons only in one quadrant. Mice were given a ten minute period without any stimulation, followed by 15 minutes in which 488 nm pulsed laser stimulation delivered to the transfected neurons via the fiber optic when the mouse entered one of the four quadrants of the test chamber. We measured the time a mouse spent in the stimulation quadrant during the stimulation period compared to the time it spent within the same quadrant during the initial 10 minutes of non-stimulation period. The effects photostimulation of channelrhodopsin transfected neurons have on approach-avoidance behavior can be measured quantitatively as the difference between the time (minutes) mice spent in the stimulation quadrant and the average of time mice spent in other three quadrants during the 10 minute testing phase, Henceforth defined as Δt. By comparing Δt values of non-laser stimulation phase to experimental laser phase, it is possible to discern the effects of photostimulation. The results showed that the activation of random ensembles of

neurons in the anterior domains of plCoA caused aversion to the stimulation, whereas the activation of neurons in the posterior domains caused attraction. We observed Δt values between 2.4975 to -1.3475. The Δt values were plotted as a function of anterior-posterior coordinates, revealing a linear relationship with a correlation coefficient of -0.6034, with the sample size of n=37 (**Figure 1a, b**). The results of this experiment reveal that there indeed is a functional, spatial organization of plCoA across anterior-posterior axis.

We validated the optical activation of neurons by assaying for the expression of the immediate early gene, c-Fos, that serves as a proxy for neural activity. Animals were exposed to light stimulation for 5 minutes, sacrificed one hour later, and the brain tissue was processed for c-Fos expression by immunohistochemistry. The analysis of c-Fos activity revealed that the activity of optically stimulated plCoA was increased by 93.14% compared to non-stimulated contralateral side, quantified by counting the cells labelled (**Figure 1d, e**).

We found that artificial stimulation of anterior and posterior regions of plCoA elicits aversion and attraction, respectively. However, activation of the structure by aversive odors such as TMT results in overall equal activation of neurons throughout the entire anterior-posterior axis, unlike that of appetitive odors which results in concentrated activation of the posterior region [1]. This implies that aversive odor would activate both aversive and appetitive neurons. Alternatively, the posterior may be a heterogeneous structure and posterior neurons responsive to aversive odor could equally drive aversive behavior. To discriminate between these possibilities, we used an approach to express channelrhodopsin in odor-activated neurons selectively in the

anterior or posterior domains. The *Arc*-CreER^{T2} mouse allows for controlled expression of channelrhodopsin, temporally through tamoxifen-dependent Cre-lox recombination, and conditional to sensory stimulation through the *Arc* immediate early gene promoter (**Figure 2 a**). A virus for a Cre-dependent channelrhodopsin-eYFP was injected into either the anterior or posterior regions of plCoA, with the fiber optic implant above the injection. One week after recovery from the surgery, tamoxifen was administered, after which the subjects were exposed to TMT (**Figure 2 b**). This enabled the conditional expression of channelrhodopsin only in the transfected neurons in either the anterior or posterior plCoA that were activated by the odor stimulation (**Figure 2 c**).

The mice were tested in the four quadrant behavioral assay as above, where they were given ten minutes without any stimulation, and 15 minutes with 488 nm laser pulse stimulation in one of the four quadrants. In the group where the anterior plCoA was targeted(n=6), the subjects showed aversion to the stimulation, with the average time spent in the stimulation quadrant decreasing from 2.259 minutes (Δt=-0.3213) to 1.782 minutes(Δt =-0.9573), a decrease of 21.11% from baseline, when the stimulation was applied (**Figure 3 a**). The analysis of c-Fos activity showed that the stimulation resulted in an increase of activity of neurons in the anterior plCoA by 78.87% compared to non-stimulated contralateral side (**Figure c**). In contrast, stimulation of the posterior TMT-responsive neurons(n=4) caused varying responses from no change in behavior to attraction. The average time spent in the stimulation quadrant increased from 2.490 (Δt=-0.0131) minutes to 3.007 minutes (Δt=0.6757) with the stimulation, an increase of 20.76% from the baseline (**Figure b**). c-Fos activity shows that the laser stimulation increased the activity of the neurons expressing channelrhodopsin by 53.94%

compared to non-stimulated contralateral side (**Figure d**). The activation of TMT-responsive neurons in anterior regions of plCoA resulted in consistent aversive behaviors, while the activation of those in the posterior region resulted in varying results with a trend towards attraction.

These results are consistent with the postulation that not all of the neurons across plCoA responding to the aversive odors contribute equally towards evoking the aversive behavior. Furthermore, the results are also consistent with the model of spatial organization of plCoA, that the anterior plCoA is wired for aversion and posterior region is wired for attraction. However, it is unclear why aversive odor should activate neurons throughout the entirety anterior posterior axis of plCoA, instead of just anterior subsection of plCoA.

The previous experiment established the sufficiency of the activation of anterior region of plCoA for aversive behavioral response, and that of posterior region for appetitive behavioral response. We next sought to test the necessity of each region for involvement in behavioral response toward innately appetitive or aversive odors. We hypothesized that the anterior should be required only for aversive responses to odor, whereas the posterior should only be required for attraction. To test this, halorhodopsin-eYFP was expressed bilaterally into the anterior or posterior region of plCoA by viral injection, with fiber implants above the injection site. The subjects were then tested in the four quadrant chamber, ten minutes without any stimulation, and ten minutes with odor stimulation in one quadrant. The animals were tested twice with TMT and twice with 2-phenylethanol, with and without 568 nm laser stimulation during the duration of the odor phase.

For the group with halorhodopsin expression in the anterior plCoA(n=5), the subjects spent on average 1.422 minutes less in the stimulation quadrant when exposed to TMT without laser stimulation, and 1.231 minutes less with the laser stimulation. On the other hand, the same animals spent 0.382 minute more in the stimulation quadrant with 2-phenylethanol exposure without the laser stimulation, and 0.282 with the laser (**Figure 4 a, c**). For the group with halorhodopsin expression in the posterior plCoA(n=4), it spent 0.975 minutes less in the stimulation quadrant with TMT without laser, 1.294 minutes less with TMT with laser, 0.158 minute more with 2-phenylethanol without laser, and 0.043 with laser (**Figure b, d**). Therefore, there was no significant change in the behavior. Analysis of odor-evoked c-Fos activity of halorhodopsin expressing neurons in plCoA shows that the photostimulation may have failed to reduce the neuronal activity (**Figure 5 c**). Rather, the c-Fos expression increased slightly by 21.56% relative that of halorhodopsin expressing neurons on the contralateral side not receiving photostimulation (**Figure 5 e**). Therefore, the behavioral results from this experiment are not conclusive.

Discussion

The results from the behavioral experiments reveal that there is a correlation between the approach-avoidance behavior and the position of activated of neurons along the anterior-posterior axis of the plCoA: activation of random neurons in the anterior plCoA causes an aversive response, whereas posterior plCoA causes appetitive response. This suggests that the circuit that processes the olfactory stimulation and produces appropriate innate behavior has a functional, spatial organization within the anterior-posterior axis of plCoA. Such mapping of the structures of brains in spatial manner in accordance to their function can be observed commonly throughout the brain, such as the gustotopic mapping of basic tastes within the primary gustatory cortex, body maps within the somatosensory and motor cortices, and coarse tonotopic map within auditory cortex [13, 14]

The first experiment utilized an experimental method where random sets of neurons, regardless of their identity, were targeted for activation. The result that random sets of neurons from posterior plCoA causes appetitive behavior is consistent with the previous finding that appetitive odors activate neurons selectively in the posterior plCoA, However, the fact that innately aversive odors also activate these neurons within posterior plCoA introduces a potential conundrum: why should aversive odors activate both populations of neurons involved in aversion and attraction? One possibility is that the posterior contains a heterogeneous set of neurons, such that aversive odor activates neurons for aversion in the posterior and attractive odor activates neurons for attraction.

To investigate further, in the second experiment, only TMT-responsive cells were selectively activated. Activation of TMT-responsive neurons in the anterior plCoA causes aversion, whereas activation of TMT-responsive cells in the posterior plCoA causes a net attraction. From this result, it can be concluded that not all TMT responsive neurons contribute equally towards innate aversion to TMT. TMT-responsive neurons in the anterior plCoA are sufficient to invoke the aversive behavior, whereas the TMT-responsive neurons in the posterior plCoA, by themselves, do not, instead causing attraction on average.

The experimental results suggest the existence of spatial segregation of neurons according to their role in the behavioral valence. Even within the subpopulation of cells selected by their responsiveness to TMT, a predator odor well established as an innately aversive odor that invokes avoidance from mice, stimulating neurons within the posterior section of plCoA does not seem to invoke avoidance behavior. One possible explanation for these observations may be that the anterior and posterior domains of plCoA have segregated projections to separate downstream structures, where the anterior domain of plCoA projects more strongly to structures associated with aversive behavior such as the medial amygdala (MeA), while the posterior plCoA may project to structures associated with appetitive behaviors such as the nucleus accumbens (NAc) that are related to rewards. Indeed, current work in the lab suggests that distinct neurons in plCoA projects to NAc and MeA and mediate approach and avoidance behavior.

Since aversive odor recruits both anterior and posterior plCoA, however, there must be an explanation as to why recruiting both domains of plCoA results in aversive

behavioral response. One possibility is that there may be a mechanism downstream of plCoA such as feedforward inhibition that allows for aversive responses to win out over the appetitive response. Evolutionarily, it may have been more important for an animal to respond to aversive cues within a complex odor landscape by avoidance. Alternatively, aversive odors such as TMT elicit a complex mixture of investigation, freezing and avoidance behaviors that can be simplified into aversion, but ultimately there could be a role for attraction during the investigation component. It would be interesting to look at the temporal sequence of activation over the time frame of behavior.

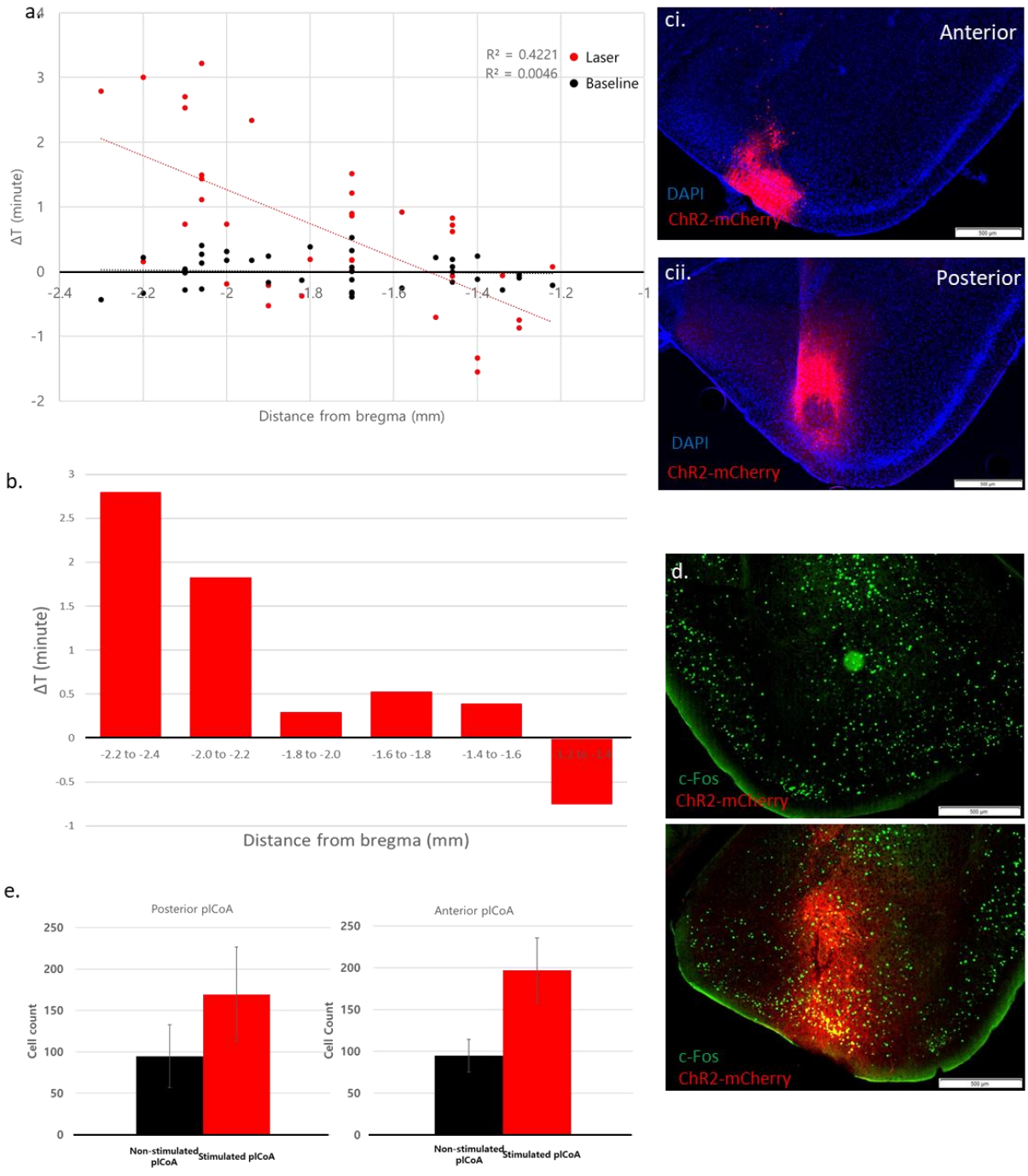
The inhibition of anterior and posterior plCoA by optogenetic methods did not yield a significant difference in behavior, as the animals did not show any change in attraction or avoidance of odor upon photostimulation of halorhodopsin expressing neurons of plCoA. Furthermore, the lack of innate attraction to 2PE renders this portion of the study impossible to interpret. Analysis of the odor evoked c-Fos activity of the plCoA through histological methods revealed that the transfected neurons of plCoA were still expressing odor-evoked c-Fos even with the laser stimulation, indicating that the neurons were not effectively silenced. This may be due to the fact that the animals were exposed to outside stimulation when they were connected to fiber optic patch cords, as the procedure requires handling of the animal, which may invoke fear response from the animal before the laser stimulation could silence the structure. Another explanation could be that the inhibitory halorhodopsin was unable to transfect significant population of cells in the target structure, which does not completely silence the target structure with the laser stimulation. Moreover, the size of the experimental

pool may have been too small at n=4 and n=5. Nonetheless, the c-Fos expression suggests that this manipulation failed, and further experiments would be required to test the necessity of anterior and posterior domains for odor responses.

In summary, the random activation of subregions of plCoA revealed the existence of functional organization of plCoA along the anterior posterior axis, with posterior subregion signals in attraction while anterior subregion signals aversion. Furthermore, specific activation of TMT responsive neurons within the subregion of plCoA indicates that while TMT odor stimulation does recruit neurons evenly across anterior-posterior axis of plCoA, anterior subsection is sufficient by itself to invoke aversive behavior, while posterior subsection by itself was not. Further studies may be needed to discern how TMT responsive neurons of posterior plCoA differs from its anterior counterpart, and how TMT specific neurons within posterior plCoA differs from non-TMT specific neurons from the same area.

Figures

Figure 1 Activation of random sets of neurons along the anterior-posterior axis of plCoA reveals an axis for valence. **a, b** Mice were tested in a 4-quadrant area where they received optogenetic stimulation in one quadrant. **a**, Behavioral response to activation of subsets of neurons plotted as a function of location along the anterior-posterior axis for animals during the baseline period (Black Dataset) and during the stimulation period (Red dataset) (n=37). Behavioral response calculated as Δt, the difference between the time (minutes) mice spent in the stimulation quadrant and the average of time mice spent in other three quadrants. **b**, bar graph shows the average Δt in different subregions of plCoA with range of 0.2 mm on anterior posterior axis. (P-value: 0.0017 for bregma-2.2~-2.0, 0.6657 for -2.0~-1.8, 0.0868 for -1.6~-1.4, 0.2776 for -1.4~-1.2, 0.1023 for -1.4~-1.2) , Representative images of anterior (**ci**) and posterior (**cii**) subregions of plCoA showing Chr2-mCherry (red) and DAPI (blue). **d** Representative images of c-Fos activity coinciding with Chr2-mCherry. **e** Quantification of c-Fos expressing cells in the anterior and posterior plCoA (n=5, 5) (P-value:- anterior=0.0588, posterior=0.1128). Error Bars indicate the standard error of mean.


a.
R² = 0.4221
R² = 0.0046
Laser
Baseline
ΔT (minute)
Distance from bregma (mm)
ci.
Anterior
DAPI
ChR2-mCherry
cii.
Posterior
DAPI
ChR2-mCherry
b.
ΔT (minute)
-2.2 to -2.4
-2.0 to -2.2
-1.8 to -2.0
-1.6 to -1.8
-1.4 to -1.6
Distance from bregma (mm)
d.
c-Fos
ChR2-mCherry
c-Fos
ChR2-mCherry
e.
Posterior plCoA
Anterior plCoA
Cell count
Cell Count
Non-stimulated plCoA
Stimulated plCoA
Non-stimulated plCoA
Stimulated plCoA

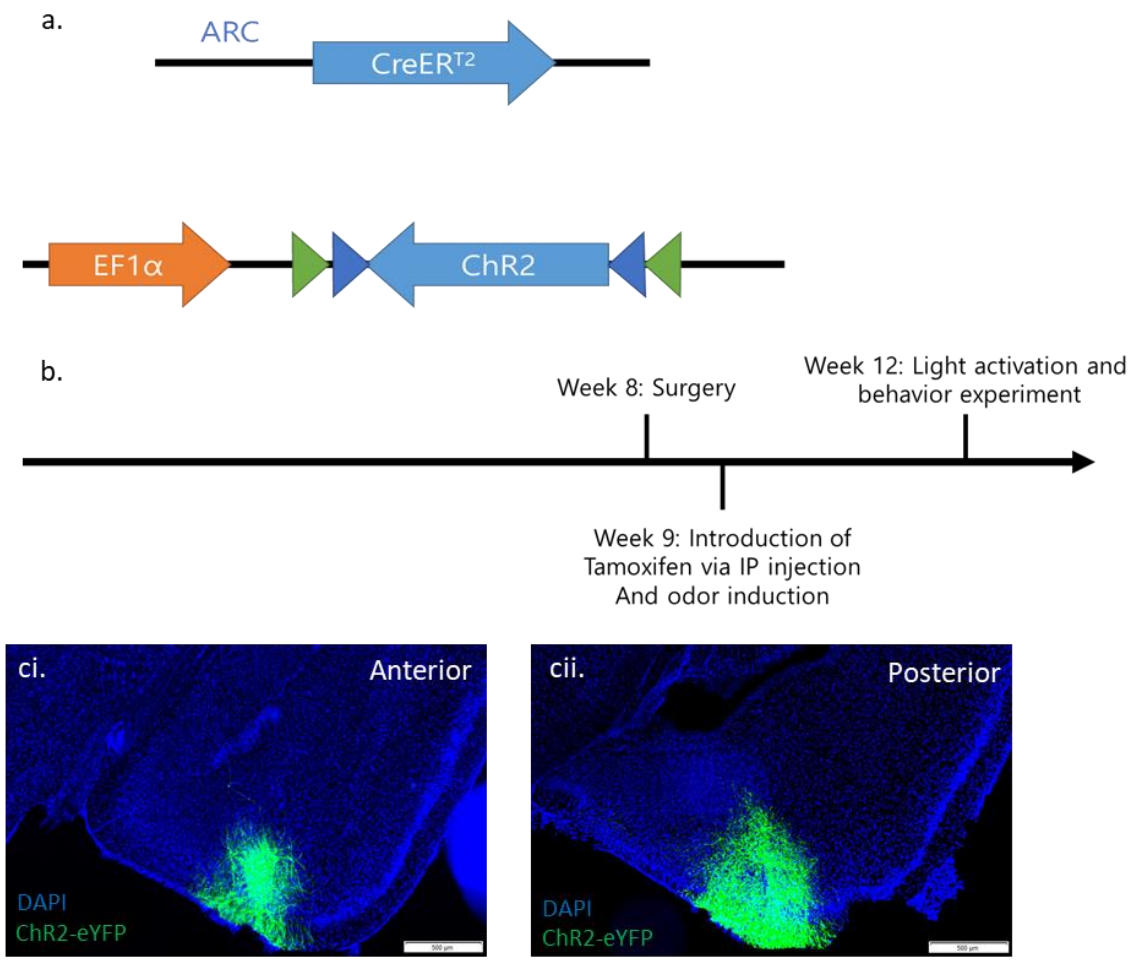


Figure 2 Labeling odor-responsive cells. *Arc*-CreER^{T2} Cre-lox system allows for temporal and conditional control of Channelrhodopsin expression. **a**, Cre recombinase CreER^{T2} is expressed by the activity-dependent *Arc* promoter. CreER^{T2} is only functional when tamoxifen is present within the system. Thus, Channelrhodopsin will only express when neurons are active and while tamoxifen is in the system. **b**, The timeline of the experiment: the mice are injected with virus and implanted with fiber at week 8, injected with tamoxifen and exposed to odor at week 9, and tested for behavior with and without photostimulation after week 12. **c**, Representative images of anterior (**ci**) and posterior (**cii**) subregion of plCoA showing DAPI (blue) and Cre-dependent channelrhodopsin-eYFP (green).

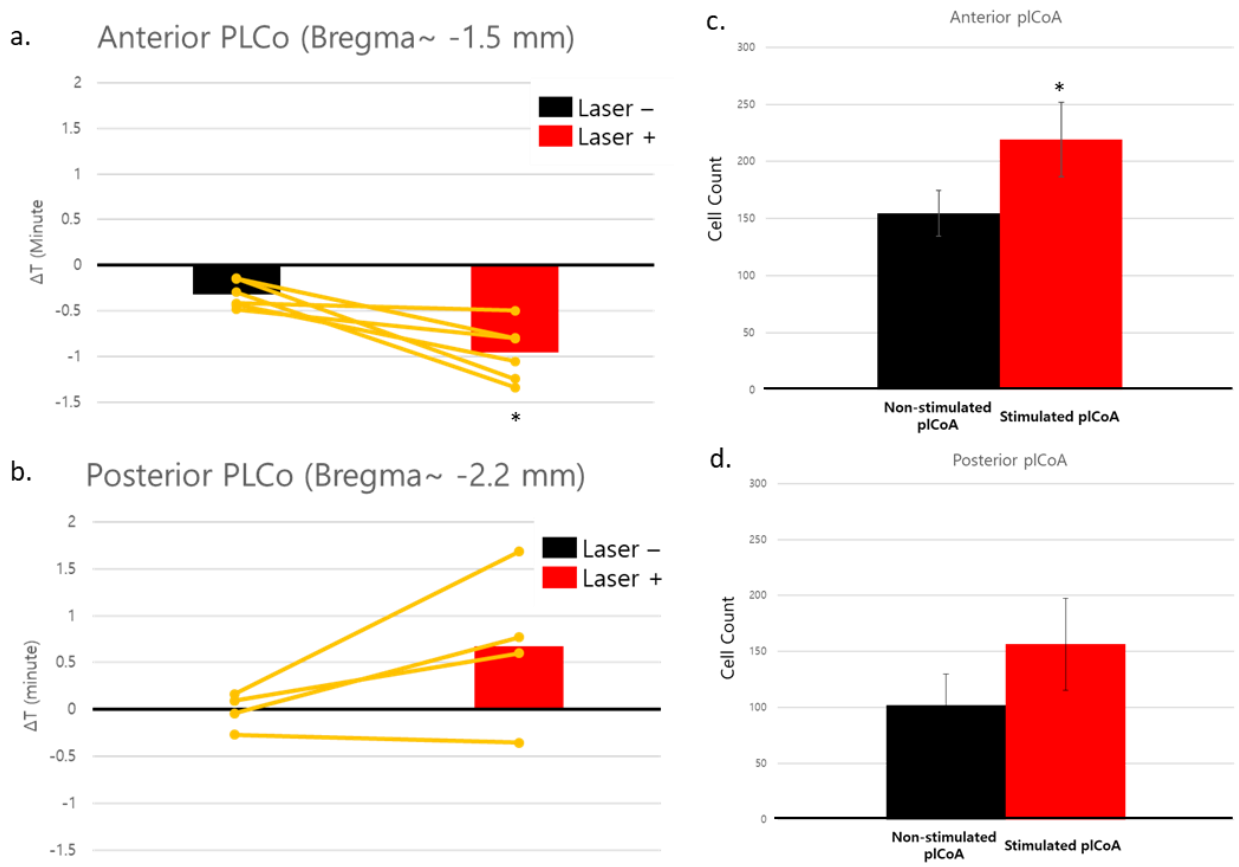


Figure 3 Activation of TMT-responsive neurons in anterior and posterior subregions of plCoA results in avoidance and attraction, respectively. Mice were tested in the 4-quadrant behavioral assay with and without optogentic stimulation **a**, Behavioral response to optogenetic stimulation of the anterior TMT-responsive neurons (n=6) (p-value=0.0112), and **b**, the posterior TMT-responsive neurons (n=4) (p-value=0.1310). Behavioral responses are quantified as Δt. **c, d** Quantification of c-Fos expressing cells after optical stimulation (n: anterior=6, posterior=4) (p-value= 0.0017, 0.0765). Error Bars indicate the standard error of mean

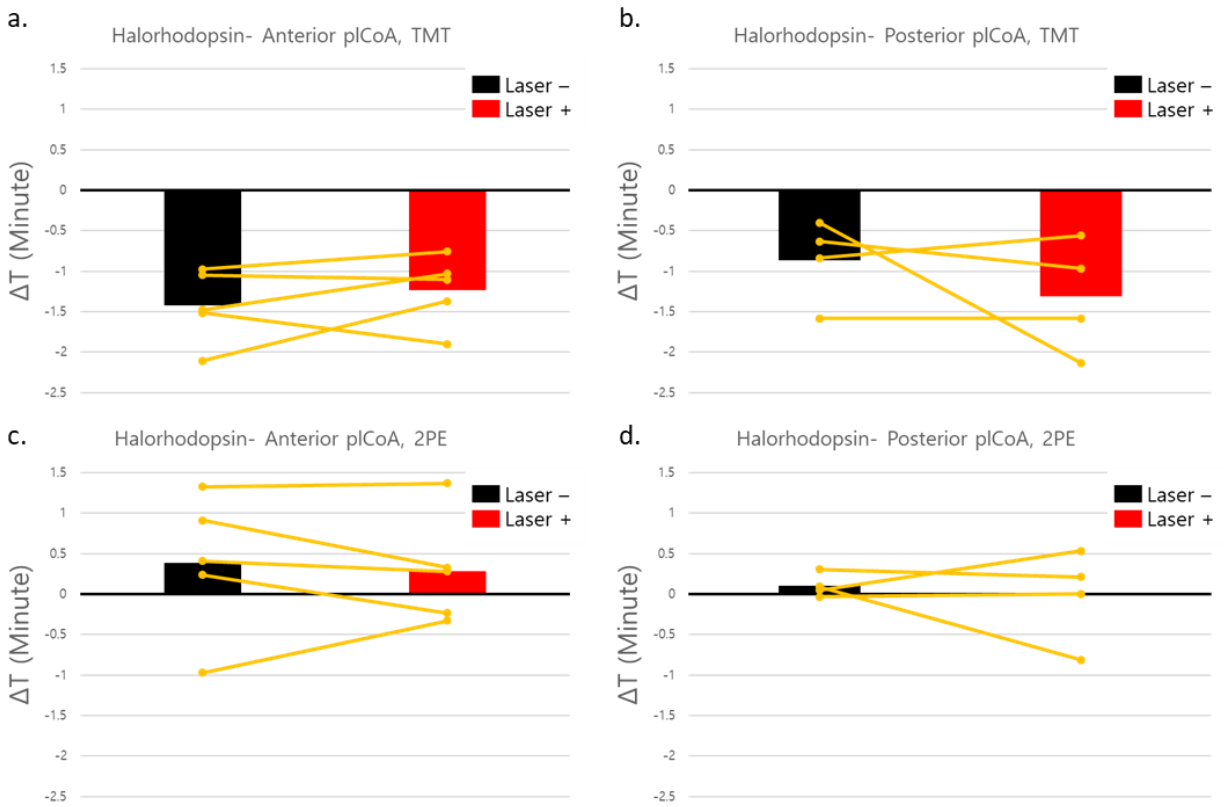


Figure 4 Attempted silencing of anterior and posterior regions did not affect odor-evoked behavior. Mice were tested in the 4-quadrant behavioral assay where they received odor in one quadrant. **a**, Avoidance to TMT with and without photostimulation of halorhodopsin expressing neurons in (**a**) the anterior and (**b**) posterior plCoA (n=5, 4) (p-value= 0.2828, 0.5969). **c**, **d**. Attraction to 2PE with and without photostimulation of halorhodopsin expressing neurons in (**c**) the anterior and (**d**) posterior plCoA (n=5 & 4) (p-value= 0.2651, 0.6211).

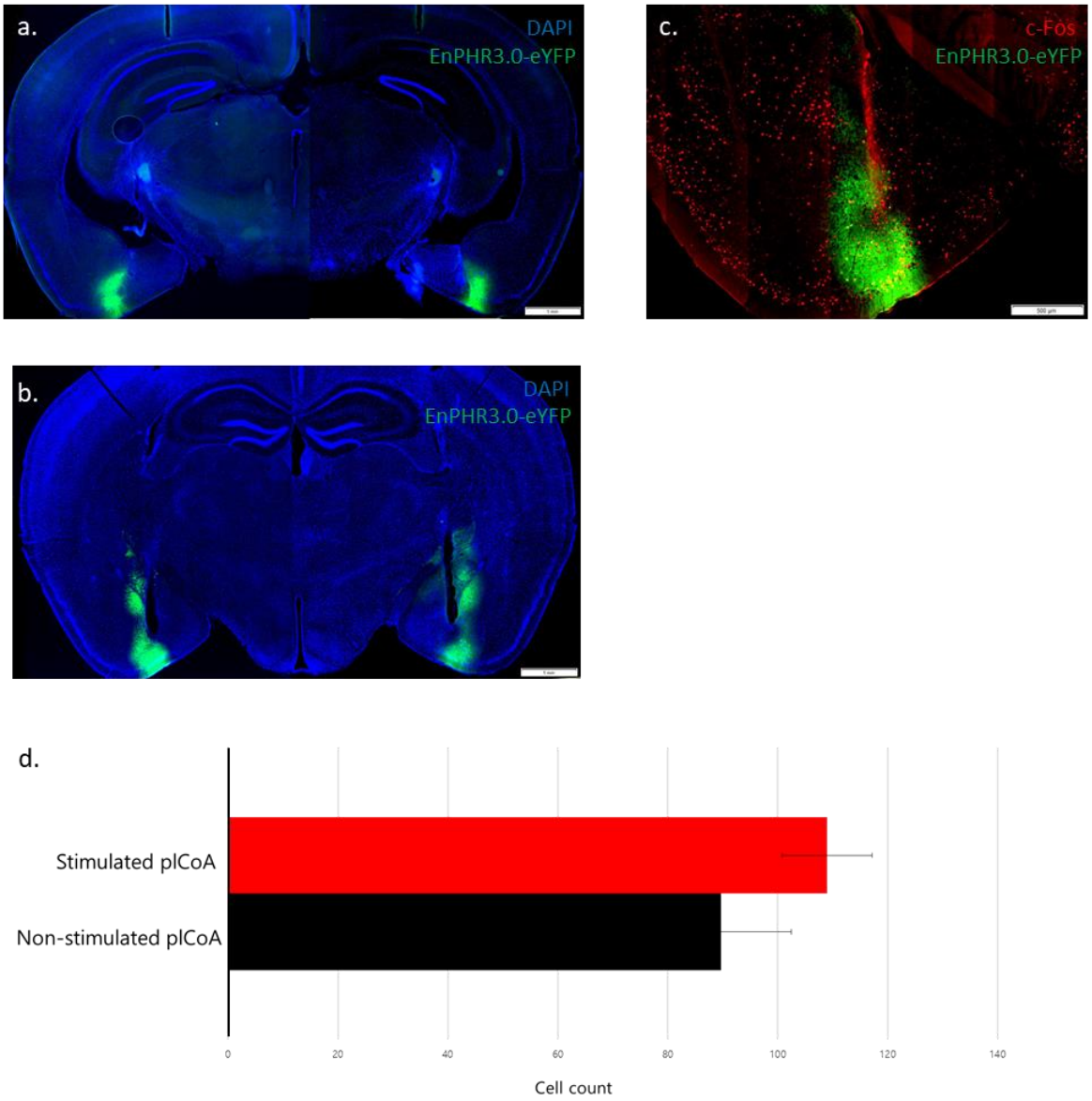


Figure 5 Analysis of odor evoked c-Fos for halorhodopsin experiment.
a, **b** Representative images of anterior (**a**) and posterior (**b**) subregion of plCoA showing halorhodopsin-eYFP (green) and DAPI (blue). **c** Representative image of plCoA expressing halorhodopsin-eYFP showing odor-evoked c-Fos (red) and halorhodopsin-eYFP (green). **d** Quantification of c-Fos experssing neurons in plCoA (n=3) (p-value=0.1796). Error Bars indicate the standard error of mean

This thesis is coauthored with Lee, James Hyeokjun, Root, Cory M., and Chan, Chung Lung. The thesis author was the primary author of this material.

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