# Plasticity of olfactory system in Drosophila melanogaster

2023.05.25 LXL JSM TW

# Plasticity



of metals was published by Henri Tresca. According to his hypothesis, metals enter a flowing state when an applied force exceeds a certain threshold. This work formed the basis for our

Tresca, 1864

TRESCA	亨利特雷斯卡 Henri Tresca	1732年 - 1807年	机械工程师	
	Henri Iresca			



# Plasticity



*Plasticity*, then, in the wide sense of the word means the possession of a structure weak enough to yield to an influence, but strong enough not to yield all at once..... Organic matter, especially nervous tissue, seems endowed with a very ordinary degree of plasticity of this sort: so that we may without hesitation lay down as our first proposition the following, that the phenomena of habit in living beings are due to the plasticity of the organic materials of which their bodies are composed. (James 1890, vol. I, p. 105; italics in the original)

James, 1890

=	Google 学术搜索	plasticity
•	文章	找到约 3,090,000 条结果 (用时 <b>0.03</b> 秒)
=	Google 学术搜索	Drosophila
•	文章	找到约 2,060,000 条结果 (用时 <b>0.07</b> 秒)
≡	Google 学术搜索	neuron
•	文章	找到约 5,410,000 条结果 (用时 <b>0.02</b> 秒)

### William James 心理学家和哲学家

What is plasticity

一种现象只有在结合了形态和功能变化的情况下才能被称为塑性。此外,这 种变化必须是持久的,即使触发这种效果的事件只是暂时的。如果形态和功 能的变化是可逆的,那么用"弹性"这个词会更合适。

# Visual and olfactory systems of Drosophila



Sugie et al., 2018, Neural Development

### Question



- What does the *Drosophila* olfactory system consist of
- ◆ What is the plasticity of the *Drosophila* olfactory system
- How plasticity in the *Drosophila* olfactory system works

### Outline

Part I: Overview of olfactory system in *D. melanogaster*—LXL

Part II: Experience-dependent plasticity in the olfactory system of *D. melanogaster* ——JSM

Part III: Molecular underpinnings of experience-dependent plasticity in the antennal lobe of *D. melanogaster* ——TW

# Part I: Overview of olfactory system in *D. melanogaster*

--LXL

- 1. Olfactory organs, receptors, and sensory neurons
- 2. The first processing center The antennal lobe
- 3. The higher brain centers The mushroom body and lateral horn

# 1.Olfactory organs, receptors, and sensory neurons



#### Benjamin Fabian et al., 2023, Front Cell Neurosci

Reinhard F. Stocker, 1994, Cell Tissue Res

## Odorant binding proteins (OBPs) promote receptor activation



Laughlin, J. D. et al., 2008, Cell

PingXi Xu et al., 2004, Neuron

### OBPs play a role in clearance of compounds from receptors to terminate responses





Elizabeth A. Scheuermann et al., 2004, Genetics

### OSNs express olfactory (ORs), gustatory (GRs) or ionotropic (IRs) receptors



Receptors	Examples	Ligands	Behavioral output
Ors	Or56a Or47b Or19a Or67d (♂) Or67d (♀)	Geosmin (toxic microbes) Methyl laurate (pheromone) Terpenes (citrus fruits) cis-Vaccenyl acetate cis-Vaccenyl acetate	Activates an aversion circuit Promotes male courtship of females Activates oviposition circuit Inhibits male-male courtship Promotes female receptivity
Antennal IRs	IR64a IR92a IR84a IR40a	Acids Ammonia and amines Phenylacetic acid (food odor) DEET	Activates avoidance responses Activates attraction Stimulates courtship on food sources Activates aversion circuit

Adult OSNs express one of about 50 odorant receptor genes and 16 ionotropic receptors

Joseph et al., 2015, Trends in Genetics

## Sensory neurons



R. Benton, 2006, Cell. Mol. Life Sci.

# 2. The first processing center – The antennal lobe



Benjamin Fabian et al., 2023, Front Cell Neurosci Grabe et al., 2015, J. Comp. Neurol

Horne et al., 2018, Elife

## OSNs expressing the same receptor type converge in the same glomerulus





#### Vosshall et al., 2000, Cell

OSNs expressing the same receptor type do not converge in the same glomerulus



Co-Receptor(s)	Total	Glomeruli
Orco, Ir8a, Ir76b, Ir25a	9	DP1I, VA3, VC3, VL1, VL2a, VM4, DL1, VA5, VA6
Ir8a, Ir76b, Ir25a	4	DP1m, VC5, VL2p, VM1
Orco, Ir8a, Ir25a	3	DL2d, DL2v, VM5v
Orco, Ir76b, Ir25a	1	VC4
Orco, Ir25a	25	DA1, DA2, DA3, DA4l, DA4m, DC1, DC2, DL3, DL4, DM1, DM2, DM4, DM6, V, VA1d, VA1v, VA2, VA4, VA7l, VA7m, VC1, VC2, VM5d, VM7d, VM7v
Ir8a, Ir25a	2*	DC4, VM6(I, m, and v)*
Orco, Ir76b	1	DC3
Ir25a	7	VP1d, VP1l, VP1m, VP2, VP3, VP4, VP5
Orco	6	D, DL5, DM3, DM5, VM2, VM3

Task et al., 2022, Elife

# The type of LNs





Tanaka et al., 2012, J. Comp. Neurol

LNs release neuropeptides, glutamate, gamma-aminobutyric acid (GABA) and acetylcholine to either inhibit or excite their postsynaptic partners



Das et al., 2011, PNAS

# The type of PNs









#### Tanaka et al., 2012, J. Comp. Neurol

## 2. The higher brain centers – The mushroom body and lateral horn



Benjamin Fabian et al., 2023, Front Cell Neurosci

# Mushroom body(MB) in Drosophila



3类: αβ, α'β', γ

- olfactory learning and memory
- context-dependent odor evaluation
- integration of different sensory modalities: olfaction, vision, gustation, thermo ,hygrosensation



Caron et al., 2013, Nature

## MB involved in innate behavior





-0.2

UAS-*shi*<sup>ts1(JFRC100)</sup> R48B04-GAL4 R58E02-GAL80

Lin et al., 2014, Nature neuroscience

Lewis et al., 2015, Current Biology

# Lateral horn(LH) in Drosophila



Das Chakraborty and Sachse, 2021, Cell and Tissue Research

Min et al., 2013, PNAS

## LH is also involved in olfactory learning and memory



Dolan et al., 2018, Neuron

Α



# Summary



# Thanks!

Part II: Experience-dependent plasticity in the olfactory system of *D. melanogaster* — JSM

# Experience-dependent plasticity in the olfactory system of *D. melanogaster*

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2023.5.25

# Contents

- 1. Introduction to glomeruli in olfactory system of *D. melanogaster*.
- 2. How does experience-dependent plasticity affect the olfactory system in *D. melanogaster*?
- 3. Improvement of experimental methods and devices.

Introduction to glomeruli in olfactory system of *D. melanogaster* 

## Location and recognition of glomeruli in *D. melanogaster*



The olfactory system of the Drosophila melanogaster adult: ~1300 ORNs(also called OSNs), 62 ORs, and ~50 glomeruli

antenna

#### maxillary palp

Fishilevich, et al. Curr. Biol. 2005

## **OR** glomerular assignments and ligand specificity

#### Α

OR	Glomerulus	Ligands
Or10a	DL1	3/28 [11, 21]: isoamyl acetate, acetophenone, methyl salicylate
Or13a	DC2	2/16 [11]: 1-octen-3-ol, 3-octanol
Or19a	DC1	11/30 [11, 21]: cineole, hexane, isoamyl acetate, pentyl acetate, ethyl butyrate, 1- hexanol, 1-octen-3-ol, 2-heptanone, 2- octanone, 1-octanol, butyl acetate
Or22a	DM2	11/29 [11, 21]: r-carvone, octanal, 3- octanone, caproic acid, hexane, isoamyl acetate, pentyl acetate, 1-octen-3-ol, ethyl butyrate, ethyl proprionate, butyl acetate
Or23a	DA3	0/16 [21]
Or33a	DA2	0/16 [11]
Or33b	DM3+DM5	0/28 [11, 21]
Or33c	VC1	1/11 [13]: E2-hexenal
Or35a	VC3I	11/17 [21]: pentyl acetate, ethyl butyrate, 1-hexanol, 1-octen-3-ol, E2-hexenal, cyclohexanone, 2-heptanone, 1-butanol, 1-octanal, ethyl 3-hydroxy butyrate, 1- nonanal, 2-pentanone
Or42a	VM7	9/30 [13, 22]: ethyl acetate, isoamyl acetate, E2-hexenal, 2-heptanone, ethyl butyrate, 1-hexanol, 2,3-butanedione, propyl acetate, 1-butanol
Or42b	DM1	11/16 [11]: cyclohexanone, r-carvone, s- carvone, linalool, 3-octanone, 3-octanol, 1-octen-3-ol, benzaldehyde, caproic acid, hexane, isoamyl acetate
Or43a	DA4	9/39 [11, 21, 23, 24]: cineole, hexane, isoamyl acetate, hexanol, 1-octen-3-ol, cyclohexanol, cyclohexanone, benzaldehyde, benzyl alcohol

Or46a	?	1/11 [13]: 4-methylphenol
Or47a	DM3	6/28 [11, 21]: hexane, isoamyl acetate, ethyl acetate, pentyl acetate, 2-heptanone, 3-(methylthio)-1-propanol
Or47b	VA1I/m	0/28 [11, 21]
Or49b	VA5	2/17 [21]: 3-methyl phenol, 2-methyl phenol
Or56a	DA2	0/16 [11]
Or65a	DL3	0/17 [21]
Or67b	VA3	13/39 [11, 22]: cyclohexanone, 3- octanone, 1-octen-3-ol, hexane, pyridine, pentyl acetate, 1-hexanol, E2- hexenal, 2-heptanone, 1-butanol, 1- heptanol, benzaldehyde, acetophenone
Or67d	DA1+VA6	N.D.
Or69a	D	1/16 [11]: isoamyl acetate
Or71a	VC2	1/23 [11, 13]: 4-methylphenol
Or82a	VA6	1/17 [21]: geranyl acetate
Or83c	DC3	N.D.
Or85a	DM5	4/30 [11, 21]: ethyl butyrate, 1-hexanol, ethyl 3-hydroxybutyrate, E2-hexen-1-ol
Or85e	VC1	3/11 [13]: ethyl acetate, cyclohexanone, (-) fenchone
Or85f	DL4	0/16 [21]
Or88a	VA1d	2/29 [11, 21]: hexane, isoamyl acetate
Or92a	VA2	3/16 [11]: r-carvone, s-carvone, octanal
Or98a	VM5	7/15 [21]: pentyl acetate, ethyl butyrate, 1-hexanol, 1-octen-3-ol, 2-heptanone, E2-hexen-1-ol, linalool



Most glomeruli express only one OR, but a few have co-expression of ORs.

## DA1 Or67d mediates behavioural responses to a Drosophila sex pheromone



How does experience-dependent plasticity affect the olfactory system in *D. melanogaster*?

### Odor exposure leads to a specific decrease in glomerular volume



BZD: 苯甲醛——DM2 and V glomerulus IAA: 乙酸异戊酯——DM6 glomerulus

Devaud, et al. J. Neurosci. 2001
## Early exposure to odor (critical period) induces experience-dependent plasticity



Devaud, et al. J. Neurobiol. 2003

## Not every olfactory circuit is affected in the same way by the odorants



The  $CO_2$ -induced plasticity is reversible.

Sachse, et al. Neuron. 2007

## Mechanism of CO<sub>2</sub> regulate the volume of V glomerulus



Flies that express both Or43a and Or83b in Gr21a OSNs  $(Or43a/Or83b \rightarrow Gr21a)$  were constructed.

Sachse, et al. Neuron. 2007

117 ± 16

360 ± 86

 $374 \pm 48$ 

 $110 \pm 35$ 

 $109 \pm 20$ 

CO2

n.s.

 $13.7 \pm 4.9$ 

 $16.4 \pm 3.7$ 

5 µm

## **Mechanism of CO<sub>2</sub> regulate the volume of V glomerulus**



The physiology of these neurons is not changed by sensory exposure.

Sachse, et al. Neuron. 2007

## The plasticity effect of VM7d glomerulus

标记突触



0-2 Days

## The plasticity effect of VM7d glomerulus

А Condition Day 3 Day 4 Day 1 Day 2 3 Day Reversal EB Dissect Oil Oil Oil 2 Day Reversal EB EB Oil Oil Dissect 1 Day 2 Day 4 Day GFP в Or42a>mCD8::GFP

Ö





The ability to undergo plasticity changes may be maintained throughout adulthood, or at least longer than previously thought, simply requiring stronger sensory stimulation.

Golovin, et al. J. Neurosci. 2019

## The plasticity effect of VM7d glomerulus



Lateral connections in AL from other activated OSNs may affect the development of VM7 during the critical period.

Odor activation of Or42a is required for remodeling of VM7 glomerulus Or42a OSNs during the critical period.

Golovin, et al. J. Neurosci. 2019

## The growth of PN dendrites is a factor that promotes the growth of glomeruli



EB: 丁酸乙酯——VM7d(VM7)、DM5、DM2 glomerulus

Chodankar, et al. J. Neurosci. 2020

## Improvement of experimental methods and devices

## Differences in glomerular PNs with/without chronic experience of odor stimulation



E2-hexenal: E2-己烯醛——DL5 glomerulus 2-butanone: 2-丁酮——VM7 glomerulus

## **Differences in glomerular PNs with/without chronic experience to odor stimulation**



## Chronic stimulation of the ORNs affects the plasticity of indirectly connected PNs



## Chronic odor exposure did not affect the sensitivity of the ORNs



## Chronic odor exposure did not affect the sensitivity of the ORNs



## Summary

- Each glomerulus expresses one or several specific classes of receptors and senses different odors.
- Odor exposure causes glomeruli to specifically decrease or increase in volume.
- Only critical period exposure to odors induces experience-dependent plasticity.
- The growth of PN dendrites is a factor that promotes the growth of glomeruli.
- Experience with chronic exposure to odors causes glomerular PNs to be more sensitive to odors at lower concentrations.



Molecular underpinnings of experience-dependent plasticity in the antennal lobe of *D. melanogaster* 

> TW 2023.5.25

# Part III: Molecular underpinnings of experience-dependent plasticity in the antennal lobe of *D. melanogaster*

--TW

- 1. Which genes are involved in the morphological changes of selective olfactory Glomeruli in *Drosophila* caused by odor exposure?
- 2. How can local GABAergic interneuron drive the habituation of plastic olfaction?
- 3. What is the Notch Signaling in the Drosophila Brain in Response to Olfactory Stimulation?

Which genes are involved in the morphological changes of selective olfactory Glomeruli in *Drosophila* caused by odor exposure?

### dunce gene

Encode a phosphodiesterase that is responsible for the degradation of cyclic adenosine monophosphate (cAMP) (Davis and Kiger, 1981)

paraffin oil (white bars) 10<sup>-1</sup>BZD(苯甲醛)(black bars) Α В volume (µm3) dunce dunce 8000+ response index -03-D DAR DAS DAG V VAZ VAZ VAZ BZD 10<sup>-3</sup> measurements test 123456 123456 10<sup>-1</sup> BZD 10<sup>-1</sup> BZD

#### 弱回避

Odor-exposed dunce mutants do not show adaptation or changes of glomerular volume

(Devaud et al, 2001)







### *rutabaga* gene

Encode a Ca<sup>2+</sup>/calmodulin-responsive adenylate cyclase, which is involved in the synthesis of cAMP (Livingstone et al, 1984 )



Glomerular volumes, neural physiology and behavior in *rutabaga* ( $rut^{2080}$ ) mutants are unaffected by long-term odor exposure

(Devaud et al, 2003)

### *rutabaga* gene

Encode a Ca<sup>2+</sup>/calmodulin-responsive adenylate cyclase, which is involved in the synthesis of cAMP (Livingstone et al, 1984 )



The effect could be rescued in rut<sup>2080</sup> mutants when functional rutabaga was specifically expressed in NP1227-Gal4 LNs

(Chodankar et al, 2020)

### *notch* gene

Encode a transmembrane receptor protein and it is involved in signal transduction between cells (Wharton et al, 1985)



How can local GABAergic interneuron drive the habituation of plastic olfaction?

### STH in Drosophila



Reduces behavioral responses mediated by these OSNs

### LTH in *Drosophila*



### cAMP Signaling is required in GABAergic LNs for habituation

and 95% of LN1-expressing interneurons А B **Short Term Habituation** LN1、GAD1、Gal80<sup>ts</sup> rescue Pre-synapse LH MB □ Naive EB Exposed (MB247-Gal4) - Post-synapse 120 Besponse Response (V PN-Gal4) (GH146-Gal4) LN 🔴 % Major classes of olfactory neurons (LN1-Gal4 / 20 and Gal4 lines that mark them GAD1-Gal4) GAL4 GAD1 LN1;TubGal80ts Or83b GH146 MB247 LN1 (Gr21a-Gal4) OSN (Or83b-Gal4) rut2080;UAS rut\* + 18°C + 29°C С D Naive Mock Long Term Habituation EB exposed 4d EB exposed Mock 4d EB exposed STH LTH 120 120 100 100 Response 80 80 60 60 40 40 % 20 LN1、GAD1、Gal80<sup>ts</sup> rescue GAD1> GAL4 GAD1 LN1: TubGal80th Or83b GH146 MB247 LN1>GAD1> LN1> LN1 +> +> ruti ruti ruti ruti ruti ruti + 18°C + 29°C rut2080 + UAS rut\*

The *rut*-encoded adenylate cyclase is required in GABAergic/LN1 neurons for habituation

RNAi-mediated knockdown of *rut* in LN1 or GAD1 neurons blocks STH and LTH to EB.

GABA-expressing谷氨酸脱羧酶(GAD1); GAD1 predominantly marks GABAergic neurons

(Das et al, 2011)

PN-specific NMDA receptors may explain odorant selectivity in habituation



The *Drosophila* NMDA receptor, a complex of NR1 and NR2 subunits, is expressed in many neurons of the adult AL







postsynaptic NMDA-type glutamate receptors on PNs contribute to such glomerular selectivity of LN potentiation.

NMDAR knockdown in EB-responsive GH146-expressing PNs

PN-specific NMDA receptors may explain odorant selectivity in habituation



LN1 terminals are an important source of both GABA and glutamate required for habituation

Habituation requires glutamate corelease from GABAergic neurons

Glomerulus-Selective Structural Plasticity Requires the Same Mechanisms Required for Odorant-Selective LTH



Expression of a WT rut+ transgene in LN1 interneurons or GAD1-expressing neurons Restores respective glomerulus-selective growth

Knockdown of NR1 in VPN neurons blocked the CO2induced increase in V glomerular volume

Knockdown of NR1 in GH146 neurons blocked LTH to EBinduced increase in DM5 glomerular volume

### CREB is required for LTH but not STH

cAMP反应元件结合蛋白 (CREB)



An inhibitory isoform of CREB, through a heat-inducible hsCREB2b transgene blocked LTH without affecting STH

CREB2b induction not only blocked LTH but also associated structural plasticity

What is the Notch Signaling in the Drosophila Brain in Response to Olfactory Stimulation?

### Monitoring Notch activation in the adult nervous system of *Drosophila*





Antennal Lobe (AL) Antennal Nerve(AN) Subesophageal Ganglion (SOG)食管下神经节

N-GV and N-LV for Notch activation

(Toby Lieber et al, 2011)

### Kinetics of Notch activation in response to odorant

To reduce any time delay in detecting the GFP signal, UAS.GFP NLS reporter(encoding a stable, nuclear localized form of GFP, rather than dGFP)



To assess the minimum time required to elicit a Notch response in ORNs how quickly Gr21a-expressing ORNs respond to CO2 exposure.

assayed for the response in nuclei of Gr21a expressing ORNs (identified by coexpression of a Gr21a.dsRed transgene)



### Notch activation by odorants depends on the DSL ligand Delta

Delta/Serrate/Lag2 (DSL) ligands and their Notch family receptors have profound and pervasive roles in development.

Two temperature sensitive Delta mutations,  $\mathsf{DI}^{\mathsf{RF}}$  and  $\mathsf{DI}^{\mathsf{6B}}$ 



Not detect an effect of heterozygosity for the weaker allele, DI<sup>RF</sup>

(Toby Lieber et al, 2011)
Notch activation depends on odorant receptor activity



An available null mutation in the gene encoding the 0r43b receptor

#### (AandB) VM2

### 表达OR43b的ORN中诱导对丁酸乙酯的Notch反应需要OR43b受体的活性

(A) dGFP in two of these classes of ORNs, those that project to glomeruli DM5 and DM6

(B) The failure of Or43b mutant ORNs to activate Notch in response to ethyl butyrate

Activation of Or43b ORNs by ethyl butyrate normally suppresses Notch activation that would otherwise occur in ORNs that innervate DM5, DM6, VA6, and D.

在任何给定的ORN中, Notch对嗅觉受体活性的反 应都可以通过其他群体中的嗅觉受体活性来调节

(Toby Lieber et al, 2011)

# Notch activation by odorants depends on synaptic transmission by the responding ORN

Using the expression of tetanus toxin破伤风毒素 To block evoked synaptic vesicle突触囊泡release in Or82a (geranyl acetate乙酸香叶酯 responsive) or Gr21a (CO2 responsive) ORNs.





在表达Or82a的ORNs中表达破伤风毒素轻链的活性(TNT)或非活性(IMP) N-LV/LexA.dGFP测定监测Notch活性 (Toby Lieber et al, 2011)

## Summary

### rutabaga gene

Both STH and LTH forms of olfactory habituation require function of the rutabaga-encoded adenylate cyclase in multiglomerular LNs that mediate GABAergic inhibition in the antennal lobe.

The odorant selectivity of STH and LTH is mirrored by requirement for NMDA receptors and GABAA receptors in odorant-selective, glomerulus-specific PNs

### *notch* gene

Notch is activated in select ORNs in an odorant-specific fashion. This response requires olfactory receptor activity and the Notch ligand Delta.

Notch activation depends on synaptic transmission by the ORNs in which the receptors are active .

