Ph.D Thesis
박사 학위논문

Early sensory-experience modulates pheromone-mediated behaviors of adult *C. elegans*

Myeongjin Hong(홍 명 진 洪 明 進)

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Advisor: Professor Kyuhyung Kim
Co-advisor: Professor Kea Joo Lee

By

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A thesis submitted to the faculty of DGIST in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Brain & Cognitive Sceiences. The study was conducted in accordance with Code of Research Ethics[1]

11. 22. 2017

Approved by

Professor Kyuhyung Kim (signature)
(Advisor)

Professor Kea Joo Lee (signature)
(Co-Advisor)

[1] Declaration of Ethical Conduct in Research**:** I, as a graduate student of DGIST, hereby declare that I have not committed any acts that may damage the credibility of my research. These include, but are not limited to: falsification, thesis written by someone else, distortion of research findings or plagiarism. I affirm that my thesis contains honest conclusions based on my own careful research under the guidance of my thesis advisor.

Early sensory-experience modulates pheromone-mediated behaviors of adult *C. elegans*

Myeongjin Hong

Accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

11. 22. 2017

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ABSTRACT

Experiences during early development can influence neuronal functions and modulate adult behaviors. However, the molecular mechanisms underlying the long-term behavioral effects of these early experiences are not fully understood. The *C. elegans* ascr#3 pheromone triggers avoidance behavior in adult hermaphrodites.

Here, I show that hermaphrodites that are briefly exposed to ascr#3 immediately after birth exhibit increased ascr#3-specific avoidance as adults indicating that ascr#3-experienced animals form a long lasting memory or imprint of this early ascr#3 exposure. ascr#3 imprinting is mediated by increased synaptic activity between the ascr#3-sensing ADL neurons and their post-synaptic SMB motor neuron partners via increased expression of the *odr-2* GPI-linked signaling and *avr-14* glutamate-gated chloride channel genes in the SMB neurons.

My study suggests that the memory for early ascr#3 experience is imprinted via alteration of a single synaptic connection, that in turn shapes experience-dependent plasticity in adult ascr#3 responses.

Keywords: Sensory imprinting, Pheromone, Synapse, GPI-anchored protein

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1. Genes related with learning and memory in *C. elegans*

	Memory for Habituation (Non-associative)		Memory for Context Conditioning (Associative)	
Gene	**Short-term**	**Long-term**	**Short-term**	**Long-term**
ins-1 [1] (Insulin)	absent	absent	normal	normal
casy-1 [2] (Type I transmembrane protein)	absent	absent	normal	N.D
hen-1 [3] (Secreted protein)	absent	absent	normal	N.D
glr-1 [4] (non-NMDA-type)	normal	absent	absent	absent
crh-1 [5] (CREB)	normal	absent	normal	normal
nmr-1 [6] (NMDA-type)	normal	normal	normal	normal
egl-4 [7] (cGMP signaling)	normal	absent	absent	absent
odr-2 [8] (Signaling protein)	absent	absent	normal	normal
magi-1 [9] (Scaffolding protein)	absent	absent	normal	normal
asic-1 [10] (Acid sensing ion-channel)	absent	absent	normal	normal
sra-11 [11] (GPCR)	absent	absent	normal	normal
tax-6 [12] (Ca^{2+} signaling)	normal	normal	normal	normal
aho-3 [13] (Hydrolase)	absent	absent	normal	normal
eat-4 [14] (Vesicular glutamate transporter)	absent	absent	normal	normal

mod-1 [15] (Serotonin-gated ion channel)	absent	absent	normal	normal
tyra-3 [16] (G-protein-coupled catecholamine receptor)	absent	absent	normal	normal
dop-3 [17] (D2-like dopamine receptor)	normal	normal	absent	absent

2. Genes related with glutamate receptor in *C. elegans*

Gene	Expression	reference
mgl-1	AIA, AIY, RMDV, RMDD and NSM	[18]; [19]; [20]
mgl-2	Interneurons	[21]
glc-3	AIY and other head neurons	[22]
glc-4	Unknown	[23]
avr-14	Expressed exclusively in a subset of 40 somatic (extrapharyngeal) neurons. Most of the neurons are in the ring ganglia of the head, but some motor neurons in the ventral cord, mechanosensory neurons	[24]
glr-1	AVA, AVB, AVD, AVE, and PVC. Also AIB, AVG, PVQ, RMD, RIM, SMD, URY	[25]
glr-2	AVA, AVD, AVE, PVC. Also in AIA, AIB, AVG, DVA, M1, RIA, RIG, RIR(?), RMDV, RMDD	[26]
glr-4	AUA, AVA (faint), AVH, DVA, DB(?), FLP, PVD, PVU (?), RIB, RIF, RIM, RMD (all), RMG, SAA (all), SAB, SIB (all), SMD (all), URA, URY	[26]

glr-5	AIB (?), AVA, AVB, AVD, AVE, AVK, DVA (?), HSN (?), LUA, PVC (?), PVQ, RIM, RIC, RIF, RME (all), RMD (all), RMG, SABVL, SABVR, SABD, SIB (all), SMD (all), URA (?), URB, URY, VC	[26]; [27]; [4]
nmr-1	AVA, AVD, AVE, AVG, PVC, RIM and in oocytes and sheath cells	
nmr-2	AVA, AVD, AVE, AVG, PVC and RIM	

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Ⅰ. INTRODUCTION

1.1 Neuroconnectome

Connectomics, the description of comprehensive maps of anatomical connection between every neuron in an organism, is a rapidly evolving field in neuroscience [28]; [29]. These large-scale wiring diagrams are expected to offer a unique opportunity to understand not only structural details of neural connectivity but also neural functions. However, previous studies demonstrated that the wiring diagrams are insufficient to decipher circuit and neural mechanisms of behavior. Thus, connecting a connectome to neural functions and eventually behaviors requires delicate physiological, anatomical and behavioral analysis at high resolution. Studies on *C. elegans* connectome and functional circuits have provided plentiful information of anatomical and functional logics of the circuit mechanisms. However, little is known about how the nervous system is functionally organized to generate behavioral outcomes.

1.2 Synaptic plasticity

Synapses are primary functional and structural units for generating behaviors. A key feature of synapses is that they are highly dynamic, and are extensively modulated by activity and experience. It is now well-established that synaptic plasticity plays a pivotal role in mediating behavioral changes in response to experience such as in learning and memory [30]; [31]. For example, learned behavioral alterations are triggered by changes in the number, structure, strength, and/or function of synapses [32]; [33]. Recent advances in connectomics have mapped the anatomical connectivities among all neurons in an organism [34]; [35]. Thus, connecting individual synapses to neuronal functions, and eventually behaviors, is a daunting challenge that requires detailed physiological, anatomical and behavioral analysis at a single synapse resolution.

1.3 Studying of *C. elegans* as a model system

The nematode *C. elegans* has a relatively simple nervous system with only 302 neurons and roughly 7,000 chemical or electrical synapses (Figure 1.1) [28].

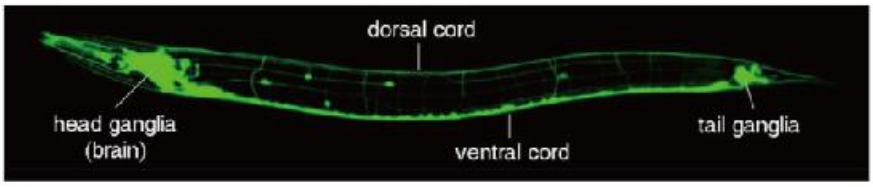

Figure 1.1 *C. elegans* nervous systems labeled by green fluorescent proteins

However, *C. elegans* displays a broad spectrum of behaviors such as locomotion, chemosensation, nociception, foraging and feeding [36]. In addition, *C. elegans* exhibits more complex behaviors including social and sleep-like behaviors, and learning and memory. For example, pairing an odor with bacterial food in a single training session results in a short-term preference for that odor, whereas pairing over multiple spaced sessions results in long-term memory that can last for 24 hr [37]; [38]. Interestingly, pairing odors with food in newly hatched animals can result in positive imprinting, with increased odor preference in adults and even in the following generation [39].

The wiring diagram or connectome of *C. elegans* has been completely reconstructed by electron microscopy (Figure 1.2) [28]; [40].

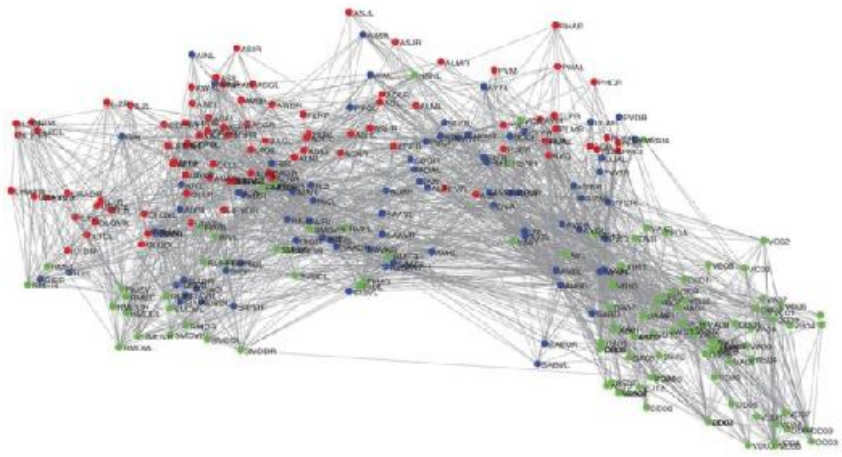

Figure 1.2 The connectome of *C. elegans* showing all 302 neurons and their chemical synapses

C. elegans has readily available genetic, genomic, and biochemical tools including opto-genetics and in vivo calcium imaging. Thus *C. elegans* can serve as an ideal organism in which to relate neuronal connectivity to whole-animal behaviors. Studies on *C. elegans* con-nectome and functional circuits have provided plentiful information of anatomical and func-

tional logics of the circuit mechanisms. Recently, the vertebrate retina has similar circuit features to *C. elegans* nervous system [41], suggesting that circuit functions are evolutionary conserved. However, little is known about how the nervous system is functionally organized to generate behavioral outcomes. More specifically, it remains still mysterious about function, chemical characteristics or activity of each individual synapse.

1.4 *C. elegans* pheromones

Pheromones are blends of released chemicals that play major roles in intraspecies chemical communication. Pheromones can elicit long-term changes in development or physiology by modulating endocrine signaling and gene expression, or short-term changes in behavior via the regulation of neuronal responses. However, their exact mechanisms in which to regulate animal behavior and development are not known yet. *C. elegans* secretes a complex cocktail of small chemicals collectively called ascaroside pheromones [42]; [43]. In the early larval developmental stage, ascaroside pheromones including ascaroside ascr#5, icas#9, ascr#2, and ascr#3 act as population density indicators to regulate growth: high pheromone concentrations cause animals to enter into the alternative non-aging, stress-resistant and developmentally arrested dauer larval stage. In the adult stage, a specific ascaroside ascr#3 acts as a sex pheromone and elicits sexually dimorphic responses; hermaphrodites are repelled whereas males are attracted (Figure 1.3) [44]; [45].

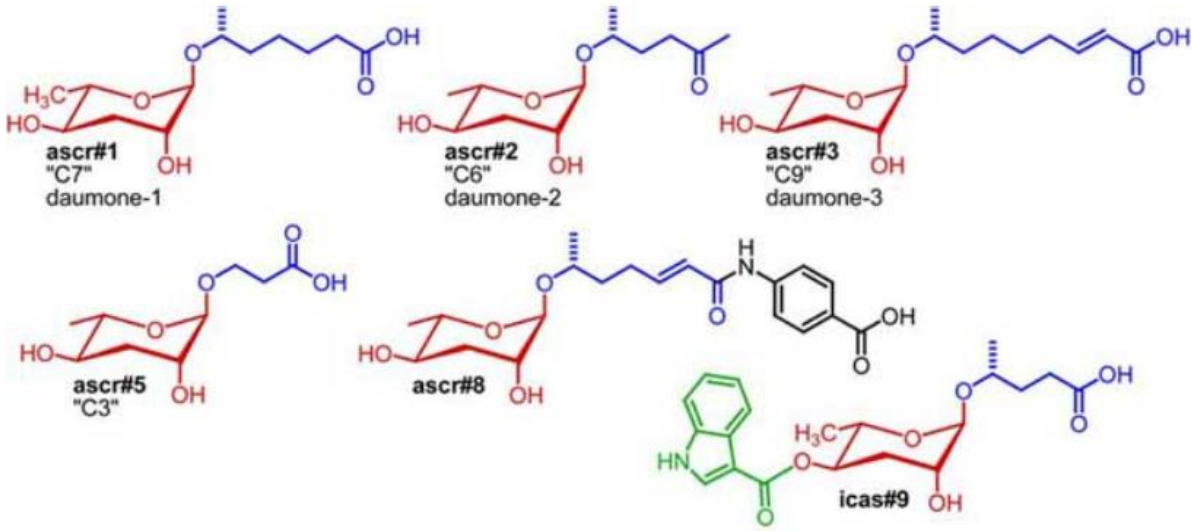

Fig1.3 Structures of ascarosides in *C. elegans*

1.5 Early pheromone-experienced study of *C. elegans*

Memories formed early in life are particularly stable and influential, representing privileged experiences that shape enduring behaviors. For example, young Pacific salmon form an olfactory memory of the natal stream that guides their return years later to spawn [46], and rodents and other mammals show a strong preference for food odors that they experience perinatally [47]. These imprinted behaviors are important for the survival and success of the animals.

The previous works identified a novel avoidance circuit to ascr#3 (asc-DC9; C9) pheromone in which the nociceptive ADL chemosensory neurons detect ascr#3 and mediate avoidance behaviors via their chemical synapses. In this research, my results show that early ascr#3 experience enhances repulsion to ascr#3 of adult hermaphrodites via the functional modification of the ascr#3 avoidance circuit, indicating that ascr#3 experienced animals form a long-lasting memory for ascr#3. To the knowledge, this would be the first example for pheromone sensory imprinting, a form of long-lasting memory. I next found that *odr-2* Ly-6-related GPI-linked gene and *avr-14* glutamate-gated chloride channel gene act in the SMB motor neurons to mediate ascr#3 imprinting. I further showed that ADL-SMB synapses are active only in ascr#3 imprinting conditions.

II. Experimental Method & Materials

2.1 Experimental Model and Subject Details

C. elegans N2 strain was used as wild-type. Wild-type and transgenic animals were maintained at 20℃ with abundant *E. coli* OP50 as food following standard conditions [48]. Day 1 (~24 hours after mid-L4 larval stage) young adult, hermaphrodite animals were used for experimentation, with the exception of the *odr-2*p*::gfp* quantification study in which the L1 stage animals were also assayed.

2.2 Method Details

2.2.1 Generation of *C. elegans* transgenic lines

odr-2 18a cDNA was amplified with a set of primers containing restriction sites, 5'-*Age*I and 3'-*Not*I using PCR and inserted into pMC10 for performing the *odr-2* rescue experiment [8]. Upstream regulatory sequences, including *odr-2* (RIG, RME and SMB), *odr-2* (-377) (RIG and RME), *flp-12* (-339) (SMB), and *lim-4* (AWB, SAA, RID, RIV, RMD and SMB),

were fused with *odr-2*cDNA to drive its expression in specific neurons [49]. To generate the *hsp::odr-2*cDNA transgene, the *hsp16.2* promoter was amplified from the *hsp16.2*p*::unc-3*cDNA transgene [50].

*avr-14*cDNA isoform b (www.wormbase.org) was amplified and inserted into pMC10 vector containing *flp-12* (-339) promoter. For analyzing the *avr-14* expression pattern, the *avr-14* promoter containing 1954 bp of upstream regulatory sequence was synthesized and fused into pPD95.75 using *Sph*I and *Xma*I restriction enzymes.

For Ca^{2+} imaging experiments of the AIB, AVD and AVA neurons, the promoter region of *sre-1*p*::GCaMP3* transgene was exchanged with *nmr-1* (AIB) or *npr-9* (AVD and AVA) promoters [51]; [52]. *nmr-1*p was amplified from pCZGY1553 (a kind gift from Yishi Jin), and *npr-9*p was 2076 bp of upstream regulatory sequence amplified from genomic DNA by PCR. These promoters were ligated with GCaMP3 using the restriction sites, *HindIII* and *Bam*HI.

Each transgenic *C. elegans* strain was generated by microinjection of the rescue construct (10 ng/μl) with *unc-122*p*::dsRed* (50 ng/μl). The *hsp::odr2*cDNA construct was injected with the *unc-122*p*::gfp* marker (50 ng/μl).

2.2.2 Pheromone imprinting

For pheromone imprinting, two types of 35 mm noble agar plates seeded with *E. coli* OP50 were made: the negative control plate contained distilled water (naive) and the experimental plate contained pheromones (pre-exposed). Figures 1K and 1L utilized experimental plates containing 600 nM ascr#3, ascr#2 and ascr#5, and 600nM ascr#3, ascr#2, ascr#5 and icas#9, respectively. All other assays in this study used experimental plates containing 600 nM ascr#3 for imprinting. To pre-expose animals to ascr#3, 5 1-day-old young adult hermaphrodites were placed onto each control and experimental plate for 2~3 hours until 30 to 50 eggs were laid. After 1 day (~26 hr), the naive or pre-exposed L1 stage animals were transferred onto 60 mm nematode growth media plates seeded with *E. coli* OP50 food and grown for 2.5 days until the young adult stage. For imprinting analysis of the next generation, 5 adults from naive or pre-exposed group (P0) were placed onto new control plates and allowed to lay 30~50 eggs (F1). The adults were then subsequently removed and the plates were incubated for 2.5 days at 20℃.

2.2.3 Post-dauer assay

Dauer formation assay was based on the protocol in [53]. 20 mL heat-killed OP50 was seeded onto 3.5mm600 nM ascr#3 noble agar plates. 510 young adults were placed and discarded when over 50 eggs were obtained. The eggs were grown at 25C for 68～72hr. Animals in dauer formation were picked onto new OP50 seeded 60 mm plates and grown at 20℃ for 2～3 days. We assayed ascr#3 avoidance with adult animals which were grown at 20℃ as control.

2.2.4 Behavioral assay

The drop assay was performed as described with modifications [52]. All behavioral assays were performed in the absence of food and used 100 nM ascr#3 diluted in M13 buffer (30 mM Tris-HCl [pH 7.0], 100 mM NaCl, 10 mM KCl). To measure the percent of animals reversing upon ascr#3 exposure, a young adult was transferred onto a plate without food and M13 buffer was dropped near the head of worm. If the worm failed to reverse in response to M13 after 10 seconds, ascr#3 was dropped onto the front of the head and avoidance behaviors (reversals) were monitored for 10 seconds. Short or long reversals were defined as reversals with fewer than two head bends or more than two head bends, respectively [54]. Long reversals were counted as repulsion in this study. The imprinting index was calculated as the percentage of reversals of pre-exposed animals minus percentage of reversals of naive animals. Each assay tested the avoidance response of 10 young adult hermaphrodite animals, and at least 3 independent trials were performed.

2.2.5 In vivo calcium imaging

Calcium imaging experiments were performed as described previously [52] using microfluidics chips that were produced in-house with a custom made chrome mask and master mold [55]. Briefly, PDMS Sylgard 184 Silicone Elastomer Kit (Dow Corning) was solidified on the master mold at 70℃ for 2 hours, and then was attached on a 24 x 24 mm glass coverslip using CUTE plasma equipment (Air) (FEMTOSCIENCE). The *C. elegans* transgenic strains used for calcium imaging experiments included *sre-1*p*::GCaMP3* (ADL), *flp-12* (-

339) p*::GCaMP3* (SMB), *npr-9*p*::GCaMP3* (AIB) and *nmr-1*p*::GCaMP3* (AVA and AVD) were used [52]. Each animal was exposed to fluorescent light for 1~2 minutes and the images were captured under fixed exposure time (100ms) for 1 min with Zeiss Axioplan microscope using a 40X objective and a Zeiss AxioCam HR. The images were analyzed by AxioVision software, Image J, and custom-written scripts in MATLAB [52].

2.2.6 Levamizole treatment

25 mM levamisole (Sigma) was used to paralyze animals. 25 mM levamisole was diluted in M9 buffer from 0.25 M levamisole. The animals were placed into the solution for about 1 minute, during which pharyngeal pumping ceased, indicating paralysis.

2.2.7 Heat shock treatment

Heat shock treatment was performed as previously described [56]. After 16 hours from egg laying, animals were twice exposed to a 33℃ heat shock for 30 minutes with a 1 hour recovery at 20℃ in between. After the second heat shock treatment, the worms were incubated at 20℃ until the adult stage and behavioral assays were performed.

2.3 Quantification and Statistical Analysis

2.3.1 Representative images

Images in Figures 4F and 4I were acquired using a Zeiss LSM700 Confocal microscope and Zeiss LSM780 Confocal microscope, respectively. The images were taken and analyzed through ZEN 2010 and ImageJ.

2.3.2 GFP quantification

For GFP quantification, the animals were anaesthetized by using 1MNaN_3 and placed on 2% agarose pad with a 24 × 24mm coverslip. *odr-2*p*::gfp* strain was integrated using UV crosslinker (UVP). A minimum of 30 *odr-2*p*::gfp* animals were exposed to 100 mM ascr#3 or control plates at the L1 larval stage following the imprinting protocol described above and

quantified for GFP fluorescence. GFP expression was scored under 40X objective Zeiss Axioplan microscopy by observing the brightness of the neuron cell body and process. The animals were observed under 40X objective Zeiss Axioplan microscopy and LSM700 Confocal microscope. The images were taken and acquired by AxioVision or Zen 2010. All strains were assayed in parallel in two independent experiments.

2.3.3 Statistical tests

All of statistics was analyzed using Prism 5.0. When only two groups were compared, two tailed unpaired Student''s t test was used. One-way ANOVA test was utilized to evaluate variation among more than two groups of datasets. The post hoc tests used include Dunnett''s test for multiple comparisons obtained at the same experimental condition, and Bonferroni''s test when the multiple comparisons were made from data produced at different conditions. All error bars indicate SEM. Detailed information of each statistical analysis is described in the figure legends.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: *C. elegans* strains		
odr-2(n2145) Ⅴ	*Caenorhabditis* Genetics Center	RRID:WB-STRAIN:CX2304
odr-2(n2145) Ⅴ; *lskEx606[odr-2Δ3*p*::odr-2*cDNA*]*	This paper	N/A
odr-2(n2145) Ⅴ; *lskEx607[flp-12Δ3*p*::odr-2*cDNA*]*	This paper	N/A
odr-2(n2145) Ⅴ; *lskEx608[lim-4*p*::odr-2*cDNA*]*	This paper	N/A

lim-4(ky403) X	*Caenorhabditis* Genetics Center	RRID:WB-STRAIN:CX3937
*lskEx218[sre-1*p*::GCaMP3; unc-122*p*:dsRed]*	This paper	N/A
odr-2(n2145) Ⅴ*; lskEx218[sre-1*p*::GCaMP3; unc-122:dsRed]*	This paper	N/A
*lskEx597[nmr-1*p*::GCaMP3; unc-122*p*::dsRed]*	This paper	N/A
*lsk Ex113[flp-12△3*p*::GCaMP3,unc-122*p*::dsRed]*	This paper	N/A
*lskEx611[sre-1*p*::TeTx; unc-122*p*::gfp]; lskEx113[flp-12△3*p*::GCaMP3]*	This paper	N/A
odr-2(n2145) Ⅴ*; lskEx113[flp-12⊿3p::GCaMP3]*	This paper	N/A
*lsk Is11[odr-2*p*::gfp;unc-122p::dsRed]*	This paper	N/A
*lskEx418[hsp::odr-2*cDNA*;unc-122*p*::gfp]*	This paper	N/A
avr-14(ad1302) I	*Caenorhabditis* Genetics Center	RRID:WB-STRAIN:CX12709
*avr-14(ad1302); lskEx894[flp-12△3*p*::avr-14*cDNA; *unc-122*p*::dsRed*]	This paper	N/A
*lskEx895 [avr-14*p*::gfp;unc-122p::dsRed]; lskEx28 [flp-12p::mcherry;unc-122p::gfp]*	This paper	N/A

*odr-2(n2145); lskEx895 [avr-14*p*::gfp;unc-122p::dsRed]; lskEx28 [flp-12p::mcherry;unc-122p::gfp]*	This paper	N/A
egl-4(ks60) IV	*Caenorhabditis* Genetics Center	RRID:WB-STRAIN:FK223
sra-11(ok630) II	*Caenorhabditis Genetics* Center	RRID:WB-STRAIN:RB816
odr-3(n2150) V	*Caenorhabditis* Genetics Center	RRID:WB-STRAIN:CX2205
odr-7(ky4) X	*Caenorhabditis* Genetics Center	RRID:WB-STRAIN:CX4
odr-10(ky32) X	*Caenorhabditis* Genetics Center	RRID:WB-STRAIN:CX32
casy-1(tm718) II	National BioResource Project	N/A
casy-1(pe401) II	*Caenorhabditis* Genetics Center	RRID:WB-STRAIN:JN414
ttx-3(ks5) X	*Caenorhabditis* Genetics Center	RRID:WB-STRAIN:FK134
tdc-1(n3419) II	*Caenorhabditis* Genetics Center	RRID:WB-STRAIN:MT13113
*lskEx610[npr-9*p*::GCaMP3; unc-122*p*::dsRed]*	This paper	N/A
Chemicals		
Levamisole	Sigma	Cat# 16595-80-5
Ascr#3		
Critical Commercial Assays		
PDMS Sylgard 184 Silicone Elastomer Kit	Dow Corning	Product code: 1064291
Software and Algorithms		
AxioVision software	Zeiss	RRID:SCR_002677
ZEN 2010 Lite Edition software	Zeiss	RRID:SCR_013672
ImageJ	https://imagej.nih.gov/ij/	RRID:SCR_003070
MATLAB	MathWorks	RRID:SCR_001622
Prism 5.0	GraphPad Software	RRID:SCR_002798

Adobe Illustrator CS6	Adobe	RRID:SCR_014198

III. Results

3.1 Adult *C. elegans* transiently exposed to ascr#3 during larval stages exhibits increased acsr#3 pheromone avoidance

3.1.1 Pheromone imprinting assay of naive and pre-exposed worms

To assess whether early experience of pheromones affects ascr#3 avoidance behavior in adult worms, I transiently exposed first larval stage (L1) wild-type worms to ascr#3 and examined their responses to ascr#3 as adults (Figure 3.1).

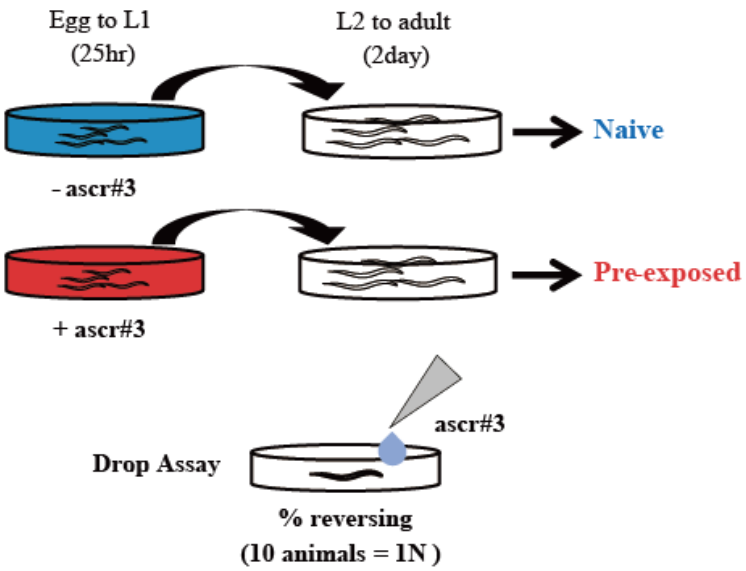

Figure 3.1 Experimental scheme of pheromone imprinting assay

To test avoidance, a pheromone diluted in buffer was applied to a freely moving animal and the fraction of animals that reverse is calculated (drop test assay) [57]. Pre-exposure to 600 nM ascr#3 during the L1 stage significantly improved adult avoidance of ascr#3 at concentrations that elicit only weak avoidance by adults that were not similarly pre-exposed, but did not further increase avoidance at higher ascr#3 concentrations (Figure 3.2).

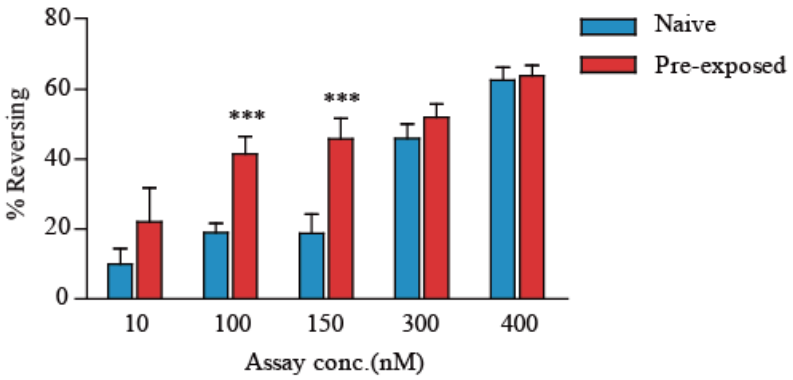

Figure 3.2 Percentage of reversal of naive and pre-exposed adult animals

Increased ascr#3 avoidance appeared to be mediated by increased long reversals and omega turns but not short reversals in pre-exposed animals (Figure 3.3) [24].

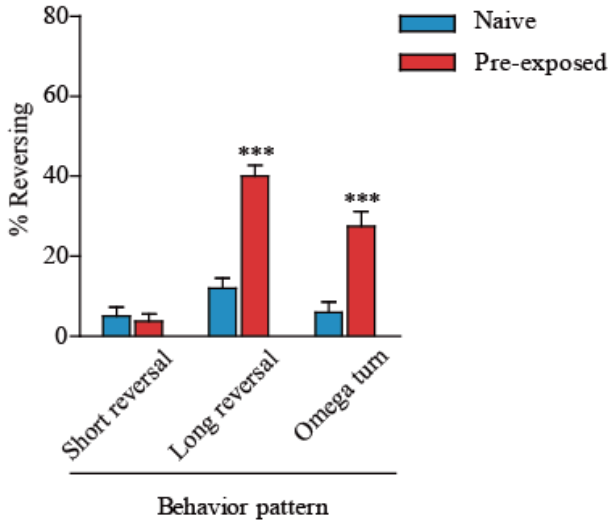

Figure 3.3 Percentage of reversal of naive and pre-exposed adult animals performing short reversal, long reversal, or omega turn

Henceforth, I calculated increased avoidance as a learning index (LI: pre-exposed reversing rate minus naive reversing rate/100) (Figure 3.4).

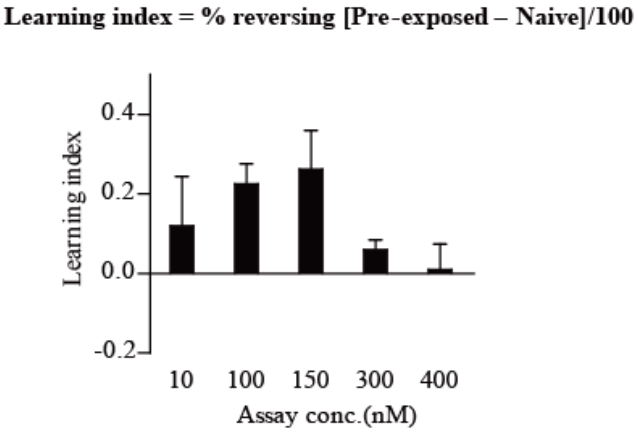

Figure 3.4 learning index of naive and pre-exposed adult animals

3.1.2 Optimal ascr#3 concentration of pre-exposure effect

I next defined the optimal ascr#3 concentration that elicits the pre-exposure effect. Animals exhibited maximal adult ascr#3 avoidance when they were pre-exposed to ascr#3 concentrations higher than 600 nM [58]; Figure 3.5).

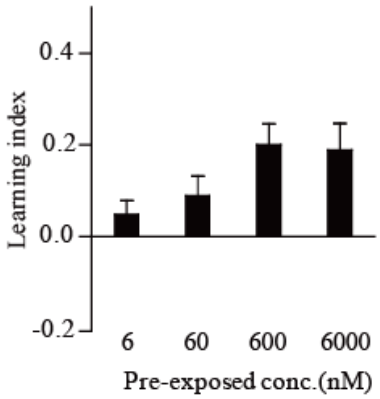

Figure 3.5 Learning index of adult animals pre-exposed to 6 nM, 60 nM, 600 nM, and 6,000 nM ascr#3 at L1

Although exposure of L1 larvae to ascr#3 pheromone also promotes entry into the alternate dauer developmental stage, I note that the conditions used for imprinting are distinct from those used for dauer induction and few if any ascr#3-imprinted animals enter into the dauer stage. These results indicate that early experience of ascr#3 pheromone appears to be translated into behavioral changes in the adult stage of hermaphrodites, suggesting that ascr#3-experienced animals form a long-lasting memory or imprint for ascr#3.

3.1.3 Early ascr#3 experience of males

Wild-type *C. elegans* males exhibit neutral responses to 100 nM ascr#3 [52]. I found that ascr#3-experienced adult males continue to exhibit neutral responses to 100 nM ascr#3 (Figure 3.6), indicating that early ascr#3 experience appears to increase ascr#3 avoidance only by adult hermaphrodites, but not by adult males.

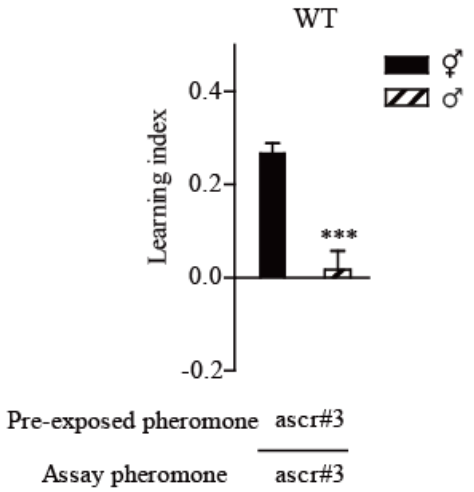

Figure 3.6 Learning index of males

3.2 The memory for the pheromone is acquired during the L1 larval stage and is specific to ascr#3

3.2.1 ascr#3 imprinting is stage specific

To determine whether the long-lasting memory for ascr#3 pheromone is formed during a specific developmental time window, worms were pre-exposed to ascr#3 at multiple developmental stages and their ascr#3 responses were assessed as adults. All assays were conducted by pre-exposing animals to 600 nM pheromone and assessing responses to 100 nM pheromone, unless noted otherwise. During the L1 stage, the *C. elegans* nervous system remodels its synaptic connections and neuronal gene expression and incorporates 80 new postembryonic neurons [59]; [60].

Compared to the L1 stage, ascr#3 pre-exposure at the L3, L4, or adult stages did not enhance ascr#3 avoidance in adults (Figure 3.7), suggesting that the L1 stage is critical for acquiring the ascr#3 memory. I could not test the L2 stage because of complexity with dauer formation [61].

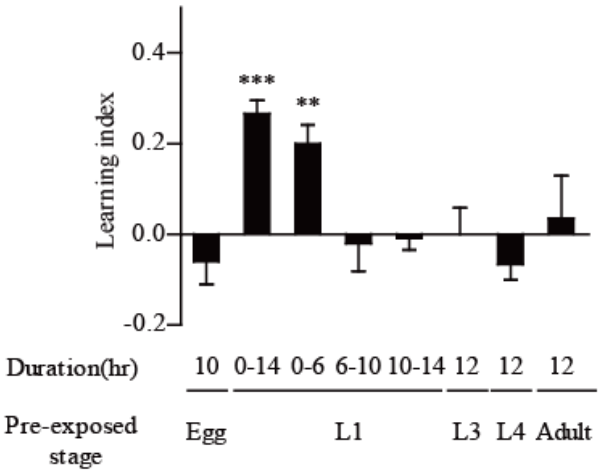

Figure 3.7 Learning index of adult animals pre-exposed to 600 nM ascr#3 at different developmental stages

3.2.2 The critical time for imprinting of L1 stages

I further defined the time window within the L1 stage necessary for this behavior, and found that pre-exposure in a defined time period during the early L1 stage (up to 6 hours

following hatching at 20 ℃) appears to represent the critical period for formation of the ascr#3 memory (Figure 3.7). More specifically, exposure of ascr#3 to worms at 2-6 hour after birth appears to be necessary and sufficient for the increased ascr#3 avoidance (Figure 3.8).

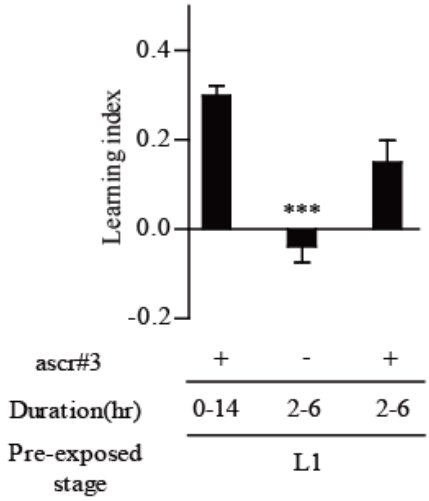

Figure 3.8 Learning index of adult animals exposed to ascr#3 for 4 hours in the L1 stage

3.2.3 ascr#3 imprinting is pheromone specific

C. elegans secretes additional pheromones, including ascr#2, ascr#5, and icas#9. Similar to ascr#3, these pheromones are also potent inducers of entry into the alternate dauer developmental stage under limiting food conditions [58]. Pre-exposure to 600nM ascr#2 or ascr#5 alone did not affect ascr#3 avoidance by adults, suggesting that pheromone imprinting does not reflect a memory of general unfavorable dauer inducing conditions but specifically encodes experience of the ascr#3 pheromone (Figure 3.9).

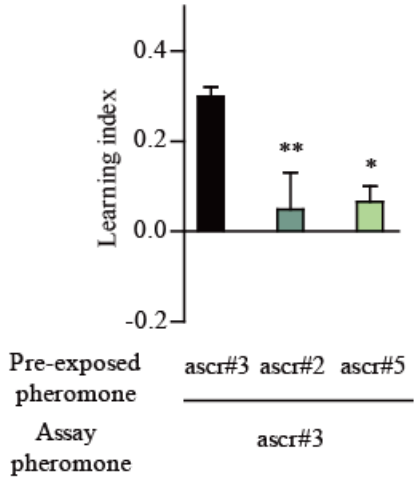

Figure 3.9 Learning index of adult animals pre-exposed and/or assayed with other pheromone components. Animals are pre-exposed to either 600 nM ascr#3, ascr#2 or ascr#5 and assayed with 100 nM ascr#3

Pre-exposure to mixtures of ascr#2, ascr#5, and icas#9 with ascr#3 also did not affect ascr#3 imprinting, further supporting that imprinting is specific to ascr#3 (Figure 3.10).

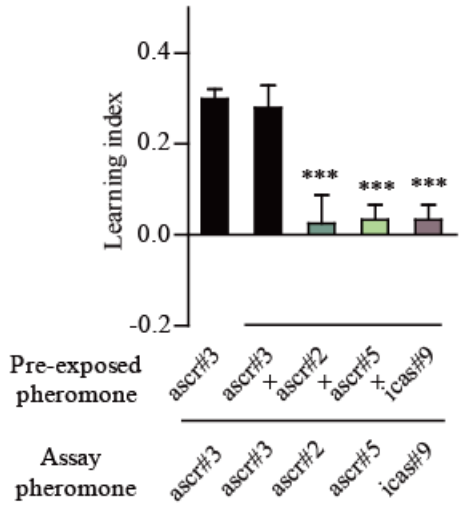

Figure 3.10 Learning index of adult animals pre-exposed and/or assayed with other pheromone components. Animals are pre-exposed to pheromone mixture containing 600 nM ascr#3, ascr#2, ascr#5 and icas#9, and assayed with 100 nM ascr#3, ascr#2, ascr#5 or icas#9

Moreover, pre-exposure to ascr#2 did not result in altered adult avoidance of ascr#2 (Fig 3.11), further confirming specificity of this process to ascr#3.

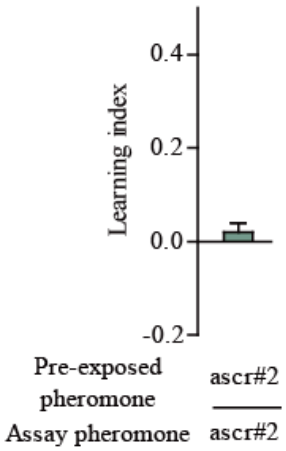

Figure 3.11 Learning index of adult animals that are pre-exposed to, and assayed with ascr#2

In addition, pre-exposure to ascr#3 did not affect high osmolality glycerol avoidance (Figure 3.12), suggesting that early ascr#3 experience does not affect broad avoidance behaviors to other repulsive chemicals by adults. However, I am unable to exclude the possibility that imprinting by other ascarosides may occur at developmental stages other than the L1 stage.

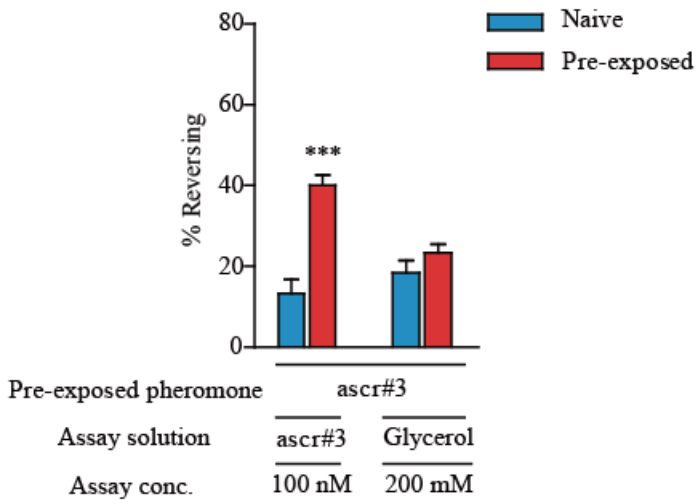

Figure 3.12 Percentage of reversal of adult animals that are pre-exposed to ascr#3 and assayed with 200 mM glycerol

Because the memory for a specific pheromone component is acquired only at the critical developmental period, I further define this altered ascr#3 avoidance as sensory imprinting [62]; [63]. Interestingly, when animals recovered from the developmentally arrested dauer stage that was induced by limited food supply in the presence of pheromones, including ascr#3, at the L1 and L2 stages, their ascr#3 avoidance was comparable to or even weaker than that of naive animals (Figure 3.13) [61].

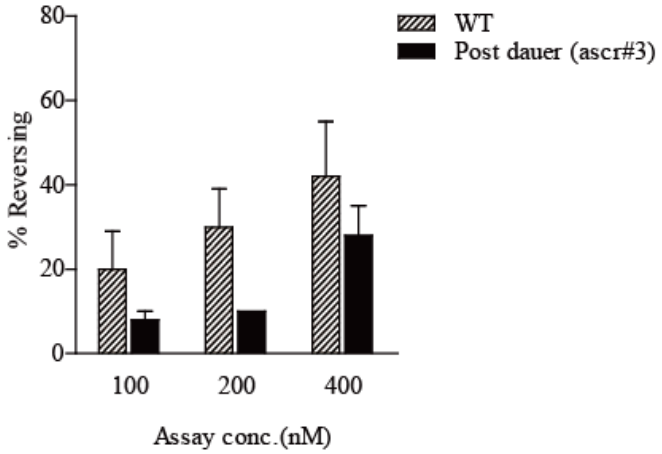

Figure 3.13 Percentage of reversal of wild-type post-dauer animals

These data indicate that the ascr#3 imprint appears to be removed following dauer experience and/or ascr#3 imprinting requires non-dauer inducing conditions at the L1 stage. Moreover, it is likely that passage through the dauer stage results in genome-wide changes in gene expression patterns [64]; [65], which may mask or erase ascr#3-imprinting phenotypes. I next investigated perdurance of the ascr#3 memory. I found that pre-exposed 3- or 6-day-old adults still exhibited increased ascr#3 avoidance (Figure 3.14).

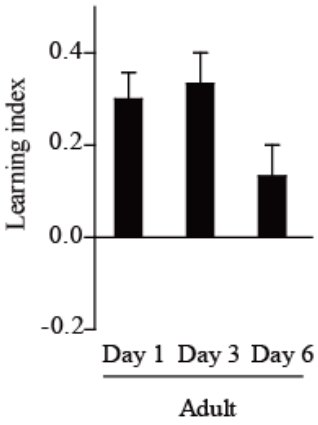

Figure 3.14 Learning index of 1, 3, or 6 day-old adults

I also asked whether memories are transmitted to the next generation but found that progeny from imprinted mothers did not exhibit improved ascr#3 avoidance (Figure 3.15). These results indicate that the ascr#3 imprint lasts with aging but is not inherited under these assay conditions.

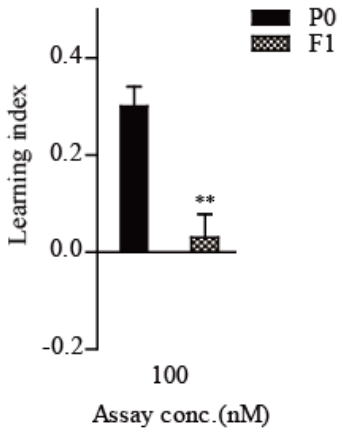

Figure 3.15 Learning index of F1 progeny of ascr#3 imprinted animals imprinting

4.1 *odr-2* acts in the SMB neurons to increase ascr#3 avoidance in ascr#3 imprinted animals

4.1.1 Candidate gene search

I next performed a candidate gene search to identify genes and molecules required for ascr#3 imprinting. I first tested genes that have been shown to be required for distinct forms of learning and memory in *C. elegans*. These include the *egl-4* (cyclic guanosine monophosphate [GMP]-dependent protein kinase) gene implicated in sensory adaptation, the *casy-1* (calsyntenin) gene involved in associative learning, and the *sra-11* (7-TM G-protein-coupled receptor), *ttx-3* (LIM homeodomain protein), or *tdc-1* (tyrosine ecarboxylase) genes involved in olfactory imprinting [7]; [11]; [66]; [67]. However, loss of function of these genes did not affect ascr#3 imprinting (Figure 4.1).

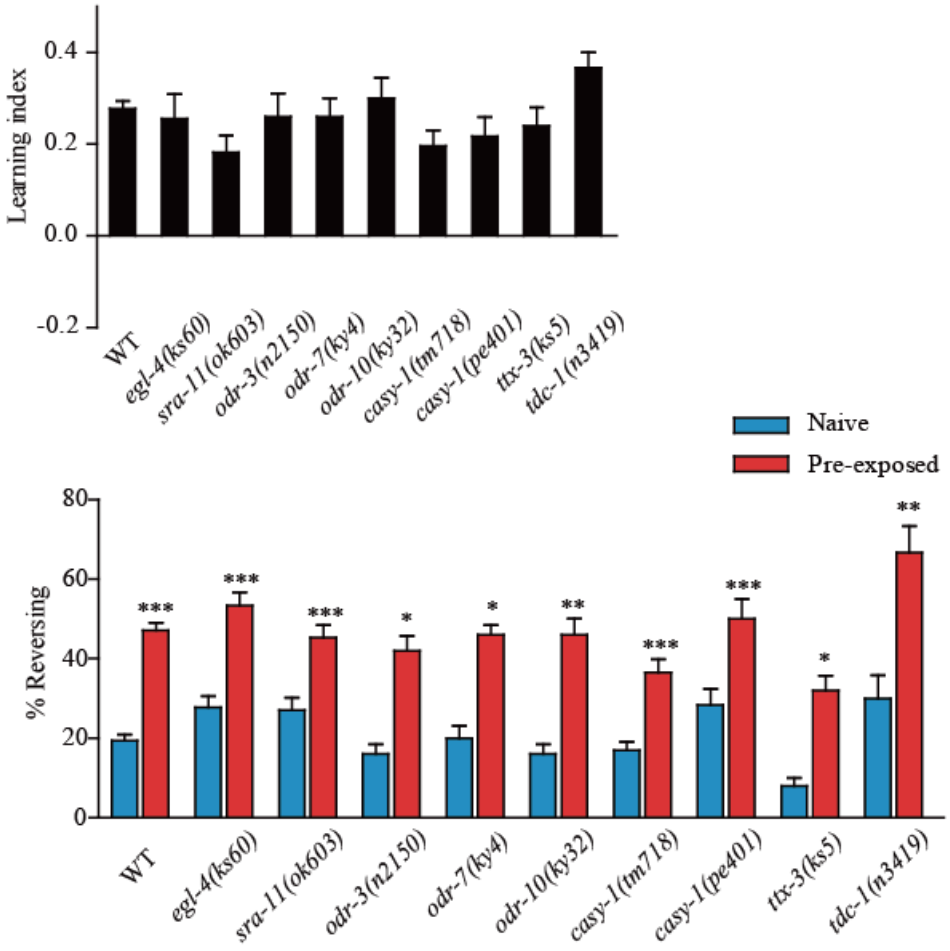

Figure 4.1 Learning index (top) and percent of reversal (bottom) of *egl-4*, *sra-11*, *odr-3*, *odr-7*, *odr-10*, *casy-1*, *ttx-3*,and *tdc-1* mutant animals

In the course of analyzing mutants defective in odorant responses, I found that *odr-2* (*n2145*) [68]; [8] mutants exhibited defects in ascr#3 imprinting although the ability of these mutants to avoid ascr#3 was unaffected (Figure 4.2, 4.3).

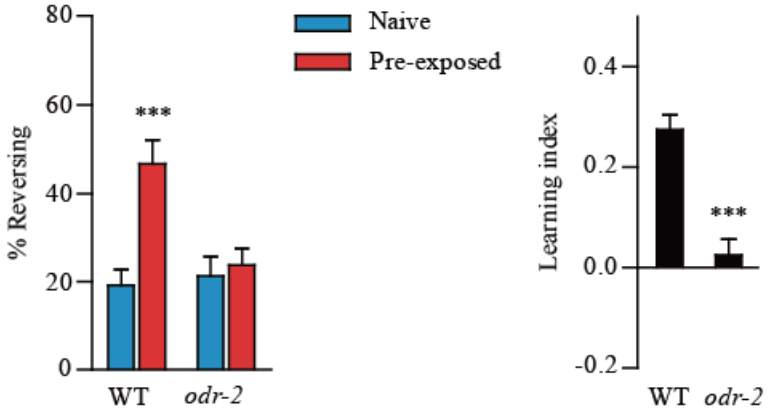

Figure 4.2 Percentage of reversal (left) and learning index (right) in wild-type and *odr-2* mutants

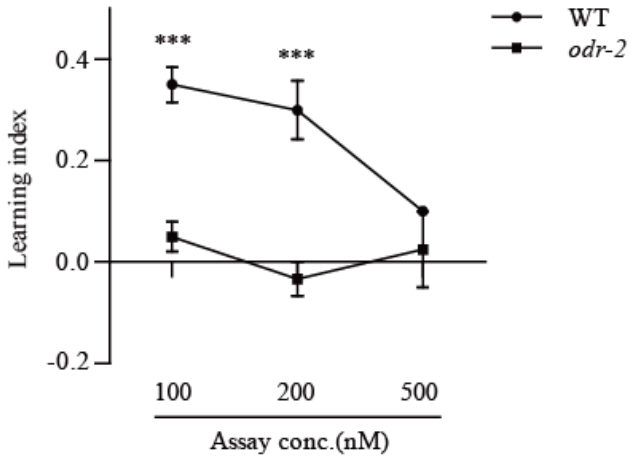

Figure 4.3 Learning index of wild-type and *odr-2* mutants at 100 nM, 200 nM, or 500 nM ascr#3

4.1.2 *odr-2* rescue study

The ascr#3 imprinting defects in *odr-2* mutants were fully rescued upon expression of wild-

type *odr-2* cDNA driven under its upstream regulatory sequences (Figure 4.4).

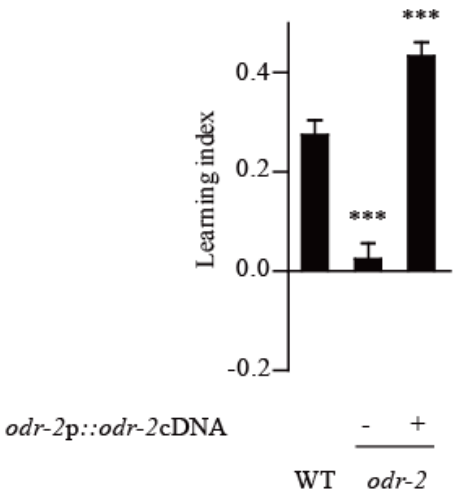

Figure 4.4 Learning index of wild-type and *odr-2* mutants expressing *odr-2* cDNA under the control of *odr-2* promoter (RIG, RME and SMB)

The *odr-2* gene encodes a membrane-associated protein related to the Ly-6 (leukocyte antigen-6) superfamily of GPI (glycosylated phosphatidylinositol)-linked proteins, and *odr-2* mutants have previously been shown to exhibit decreased chemotaxis towards a set of volatile attractive chemicals [69]; [70]; [71]. Ly-6 proteins share sequence similarity in a domain of z75 amino acids, defined by the conservation of 10 cysteine residues with a characteristic spacing pattern [72, 73] ; [74]; [75]; [76]; [77]; [78]; [79]; [80]. The predicted open reading frame (756 bp) of the salmon olfactory imprinting-related gene encodes a protein of 252 amino acids and shares low amino acid sequence identity with the urokinase-type plasminogen activator receptor (u-PAR) [81]. u-PAR belongs to a member of the Ly-6 superfamily that is found in several species [82]; [83]; [84]. *odr-2* is expressed in a set of head neurons including RIG, RME and SMB [8]. To determine where ODR-2 acts to regulate ascr#3 imprinting, I tested transgenic animals expressing *odr-2* wild-type sequences for rescue of the ascr#3 imprinting defects. I found that while expression of *odr-2* in RIG and RME did not rescue these behavioral defects, ascr#3 imprinting defects were fully restored upon expression of

odr-2 in SMB (Figure 4.5), indicating that ODR-2 acts in the SMB neurons to mediate ascr#3 imprinting.

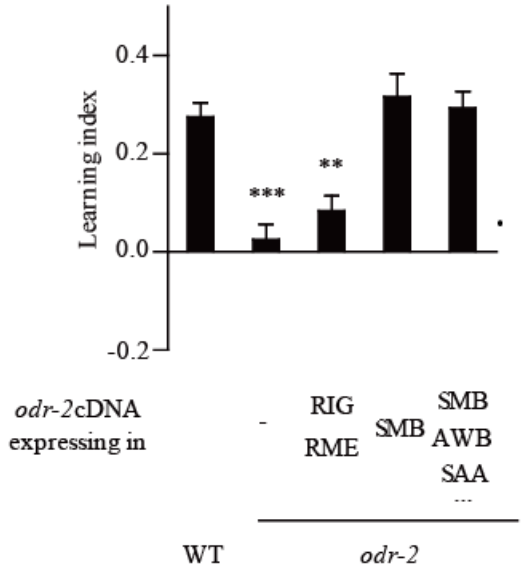

Figure 4.5 Learning index of wild-type and *odr-2* mutants expressing *odr-2* Cdna under the control of cell-specific promoters, including *odr-2* (-377) (RIG and RME), *flp-12* (-399) (SMB), and *lim-4* (AWB, SAA, RID, RMD, and SMB)

4.1.3 SMB sensory/inter/motor neuron

The SMB neurons consist of two left and right pairs (dorsal or ventral) of sensory/inter/motor neuron types that are located in the head, and that innervate the head and neck muscles [28]; Figure 4.6).

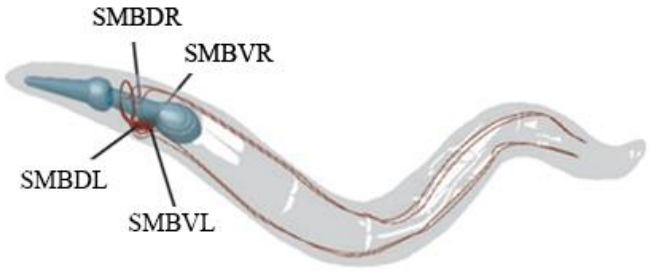

Figure 4.6 Schematic diagram of the SMB neurons

While their synapse-free processes extend along the ventral or dorsal sublateral cords to the tail, the SMB neurons have extensive electric and chemical synaptic contacts to other neurons in the head [28]. These neurons are proposed to be body stretch sensors and regulate head locomotion [28, 49, 85]. However, their roles in chemosensory behaviors have not been explored.

4.1.4 Association of SMB and Imprinting

I next investigated that the SMB neurons are required for ascr#3 imprinting. To address this issue, I examined *lim-4* mutants in which the SMB neurons are not fully differentiated and in which the functions of SMB are completely abolished [49]. While *lim-4* mutants were still able to avoid ascr#3, pre-exposure to ascr#3 did not enhance ascr#3 avoidance in *lim-4* mutants (Figure 4.7).

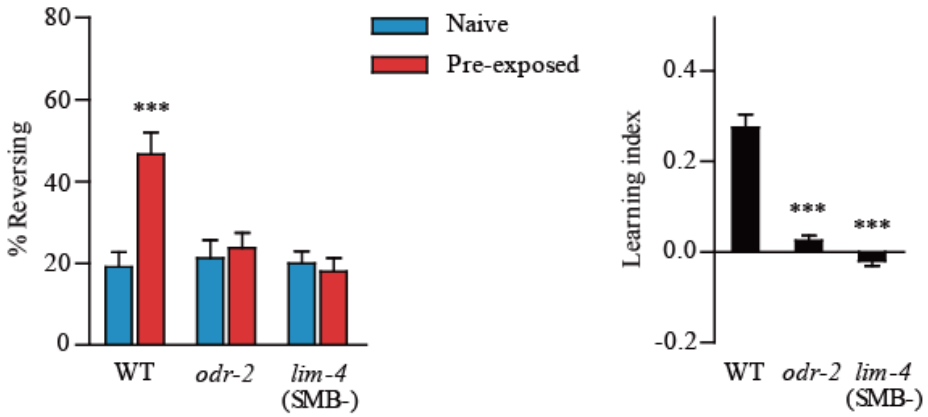

Figure 4.7 Percentage of reversal of naive and pre-exposed animals (left) and learning index (right) in wild-type, *odr-2*, and *lim-4* mutants

These results indicate that the SMB neurons are necessary for ascr#3 imprinting.

4.2 ascr#3-induced responses in the ADL chemosensory neurons are unaltered in ascr#3 inprinted animals

4.2.1 ADL Ca^{2+} imaging

In adults, ascr#3 elicits avoidance behavior in hermaphrodites via the nociceptive ADL chemosensory neurons ([52]; Figure 4.8).

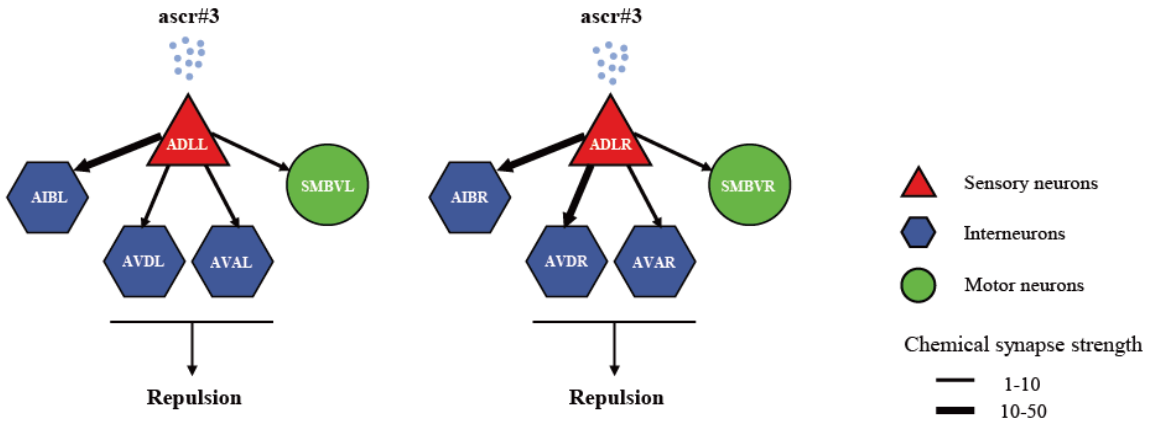

Figure 4.8 Post-synaptic connections of the ADLL and ADLR neurons. The AIB, AVD, AVA and SMBV neurons are post-synaptic to ADL

To describe the neuronal basis of pheromone imprinting, I first monitored intracellular Ca^{2+} dynamics in response to ascr#3 in transgenic animals expressing the genetically encoded calcium sensor GCaMP3 in the ascr#3-sensing ADL neurons. The ADL neurons exhibit a rapid and transient Ca^{2+} increase upon exposure to nano-molar concentrations of ascr#3 ([52]; Figure 4.9).

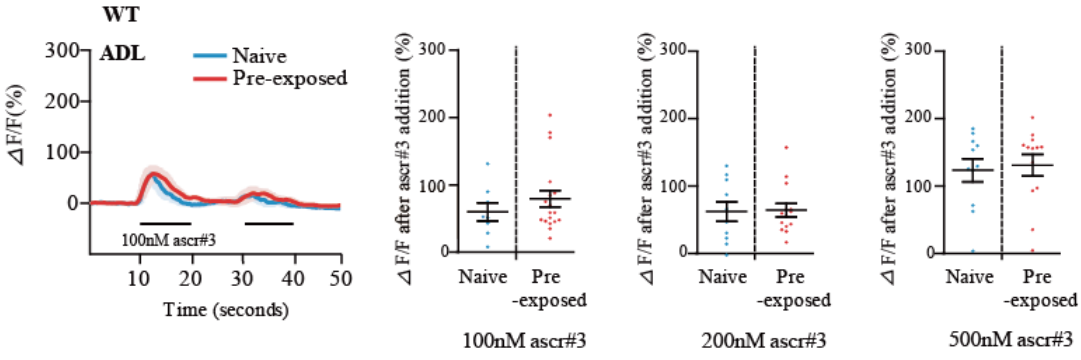

Figure 4.9 Ca^{2+} transients of ADL in response to 100 nM ascr#3 exposure

Both naive animals and ascr#3-imprinted animals displayed similar Ca^{2+} transients in adult ADL neurons upon addition of ascr#3 (Figure 4.9). These results suggest that increased ascr#3 avoidance is not due to enhanced sensory responsiveness of ADL.

4.2.2 ADL Ca^{2+} imaging in *odr-2*

Furthermore, consistent with the normal ascr#3 avoidance behavior of *odr-2* mutants, the ADL Ca^{2+} response to ascr#3 was not altered in *odr-2* mutants (Figure 4.10).

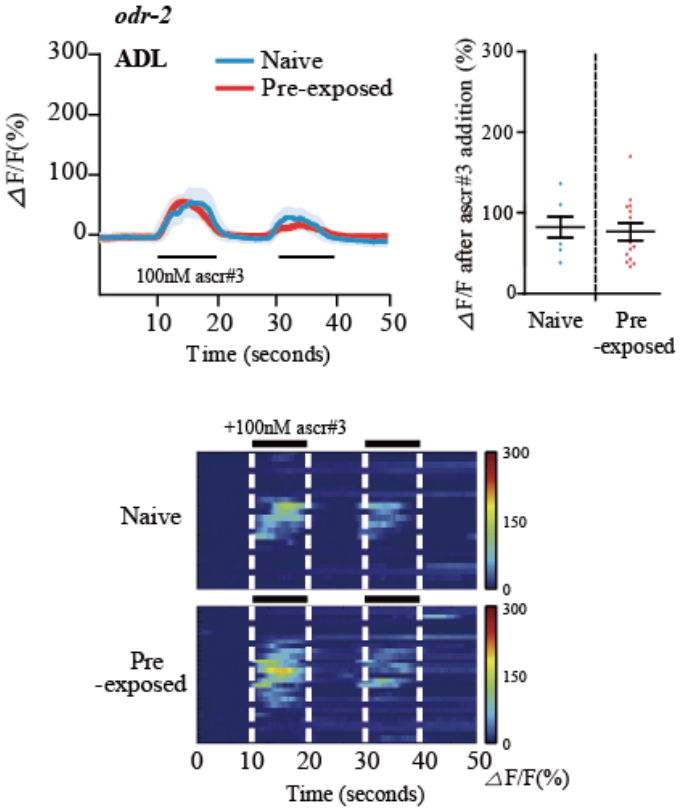

Figure 4.10 Ca^{2+} transients of ADL in response to 100 nM ascr#3 exposure in *odr-2* mutants

4.2.3 AIB, AVD, AVA Ca^{2+} imaging

The ADL sensory neurons drive ascr#3 avoidance through their chemical synapses [52]. Inspection of the anatomical wiring data from the *C. elegans* connectome suggests that ascr#3 signals from the ADL neurons are transmitted to a few major postsynaptic target neurons, including AIB (1st layer interneurons), and the AVA and AVD backward command interneurons (Figure 4.8). Previous studies have shown that activity in AIB, AVA and AVD is increased during backward movement or reversals [86]. I found that while the AIB or AVD neurons exhibited noisy but low levels of Ca^{2+} responses upon exposure to ascr#3 (Fig 4.12, 4.13), the AVA cell bodies responded strongly and consistently to repeated ascr#3 addition with increased Ca^{2+} levels (Figure 4.11).

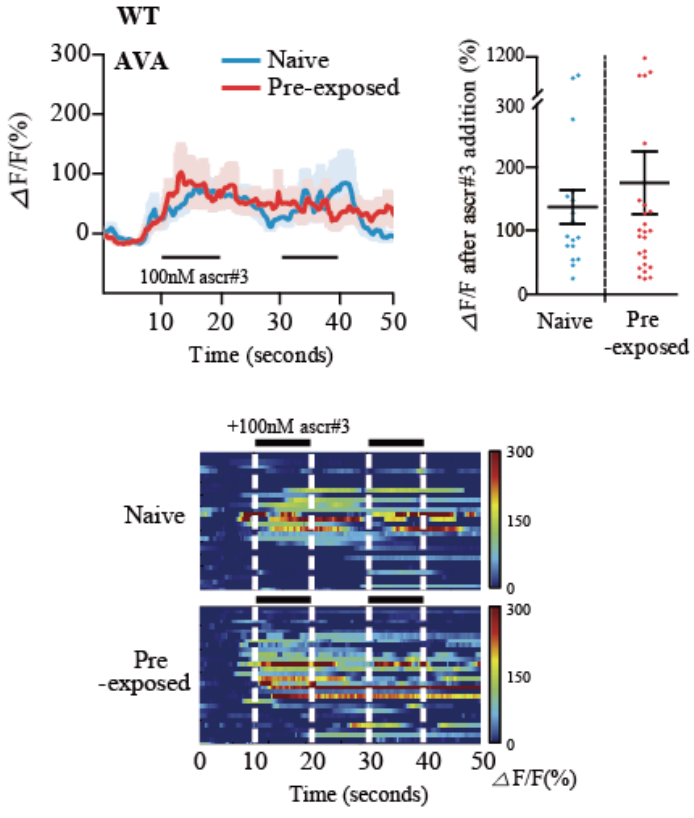

Figure 4.11 Ca^{2+} transients of AVA in response to 100 nM ascr#3 exposure

However, these responses were unaltered upon ascr#3 imprinting (Figure 4.11, 4.12,

4.13).

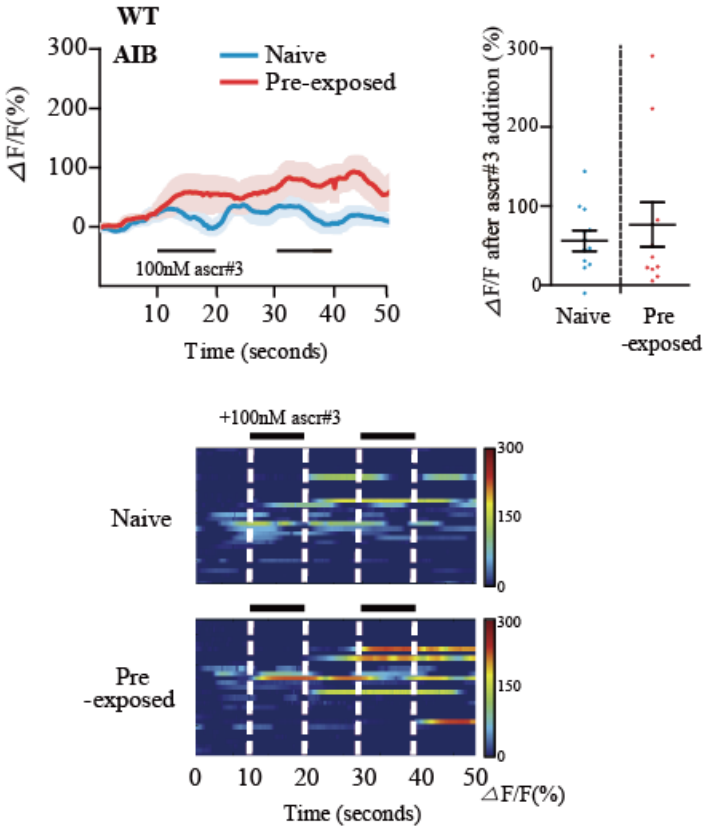

Figure 4.12 Ca^{2+} transients of AIB in response to 100 nM ascr#3 exposure

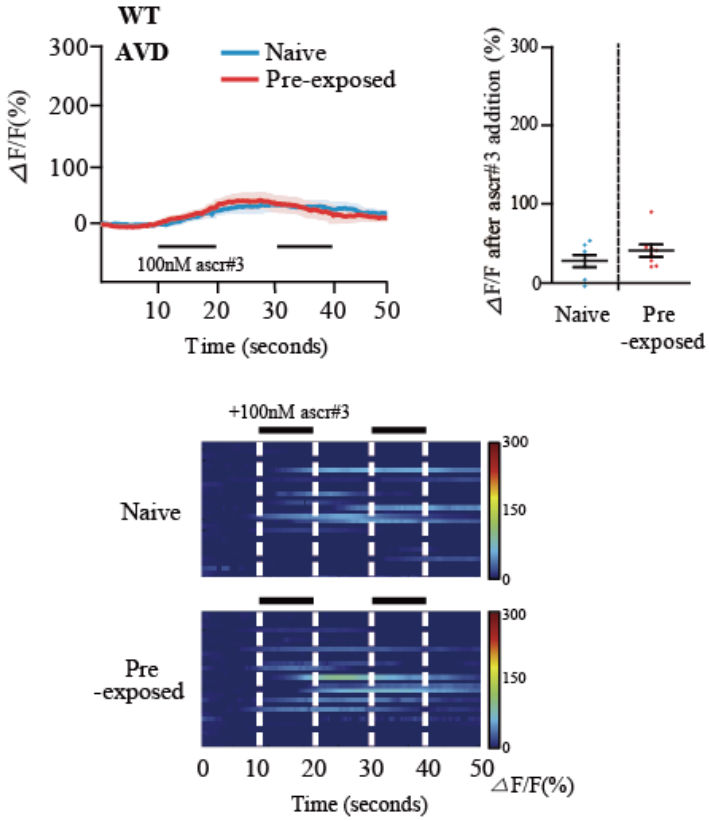

Figure 4.13 Ca^{2+} transients of AVD in response to 100 nM ascr#3 exposure

This result implies that ascr#3 signals in the ADL neurons may be transmitted mainly via AVA, but that imprinting does not enhance ascr#3 avoidance via this circuit.

4.3 SMB mediates increased ascr#3 avoidance in ascr#3 inprinted animals

4.3.1 SMB Ca^{2+} imaging

The *C. elegans* connectome data indicate that the SMBV neurons are additional post-synaptic partners of the ADL sensory neurons (Figure 4.8), although the synaptic strength appears to be weak. Because the SMB neurons appear to play a role in ascr#3 imprinting, I then examined how SMB is involved in ascr#3-avoidance behavior.

I first expressed GCaMP3 in SMB under the control of *flp-12* (-339) promoter that drives

transgene expression in all four SMB neurons [49]. I note that a SMBV-specific promoter is not currently available. Moreover, because the cell bodies of SMBD and SMBV are located in close proximity, differentiating among these neurons is challenging. I thus examined ascr#3 responses in all SMB neurons together. Acute ascr#3 exposure did not elicit Ca^{2+} transients in the SMB neurons of naive control animals (Figure 4.14). However, I detected consistent and robust Ca^{2+} transients in response to acute ascr#3 exposure in ascr#3-imprinted animals (Figure 4.14).

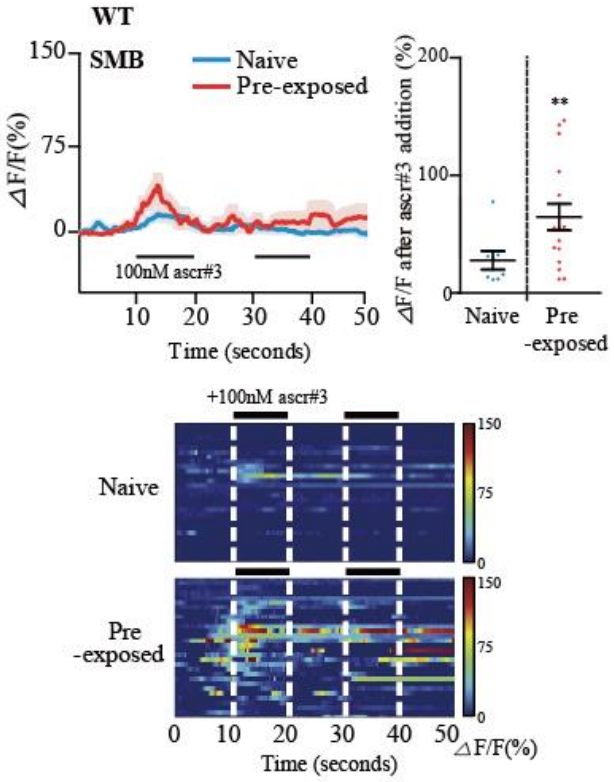

Figure 4.14 SMB mediates enhanced ascr#3 avoidance in ascr#3-imprinted animals

4.3.2 Levamisole-treated SMB Ca^{2+} imaging

Because SMB may detect body muscle contractions that could be responsible for the observed Ca^{2+} responses in SMB, I paralyzed worms with the nicotinic acetylcholine receptor

agonist levamisole. Levamisole-treated ascr#3-imprinted animals still exhibited similar or even enhanced Ca^{2+} dynamics upon ascr#3 exposure (Figure 4.15).

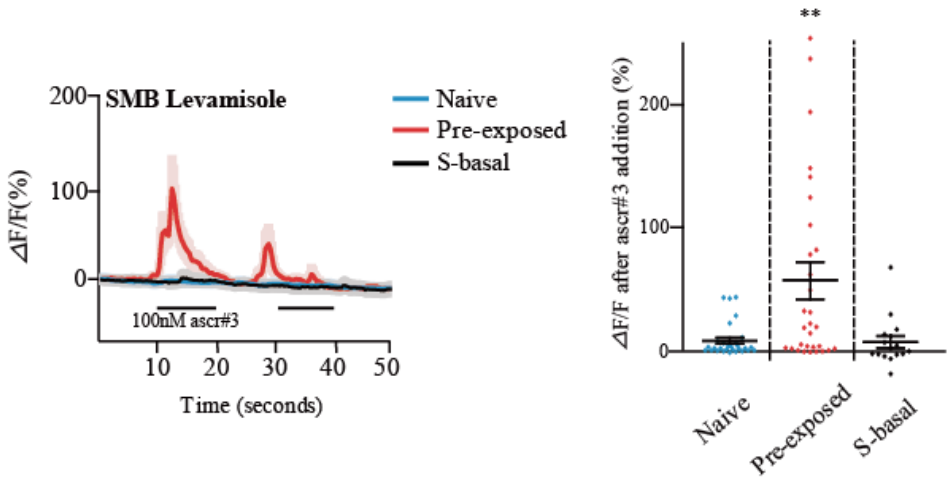

Figure 4.15 Ca^{2+} transients of the SMB neurons in response to 100 nM ascr#3 in wild-type animals treated with levamisole

4.3.3 ADLp::*TeTx*-treated SMB Ca^{2+} imaging

To test whether the observed Ca^{2+} transients in SMB in ascr#3 imprinted animals are transmitted from ADL, I examined Ca^{2+} responses in transgenic animals expressing TeTx (tetanus toxin light chain) specifically in the ADL neurons to block synaptic transmission from ADL [52]. Ca^{2+} transients of SMB in ascr#3 imprinted animals were strongly suppressed by blocking ADL synaptic transmission (Figure 4.16, 4.17), suggesting that ascr#3

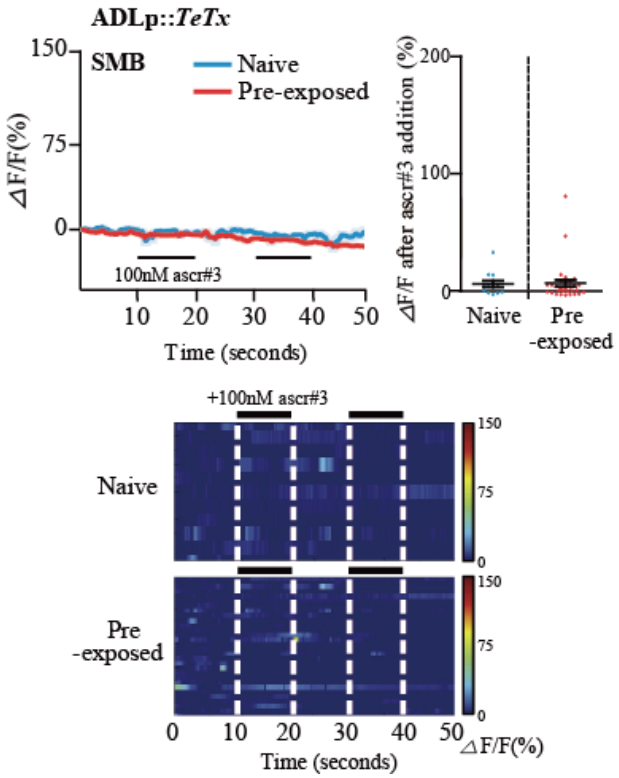

Figure 4.16 Ca^{2+} transients of the SMB neurons in response to 100 nM ascr#3 in wild-type animals expressing ADLp*::TeTx*

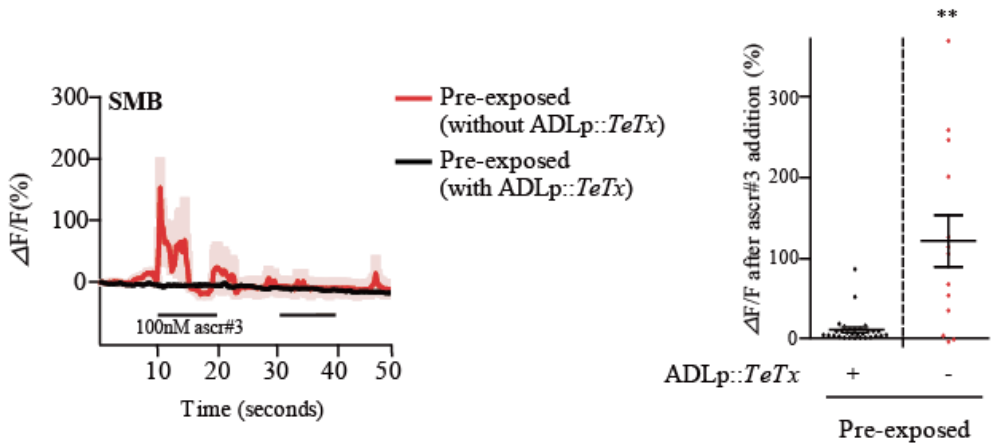

Figure 4.17 Ca^{2+} transients in SMB of animals expressing ADLp*::TeTx*

imprinting results in enhanced ascr#3 signal transmission from ADL to SMB. These results indicate that imprinting specifically alters the ADL-SMB synaptic transmission.

4.3.4 SMB Ca^{2+} imaging in *odr-2*

Moreover, the ascr#3 imprinting-dependent Ca^{2+} response in SMB was completely abolished in *odr-2* mutants (Figure 4.18), indicating that *odr-2* elicits increased activity of SMB to ascr#3 exposure in imprinted animals by enhancing the ADL-SMB synaptic transmission.

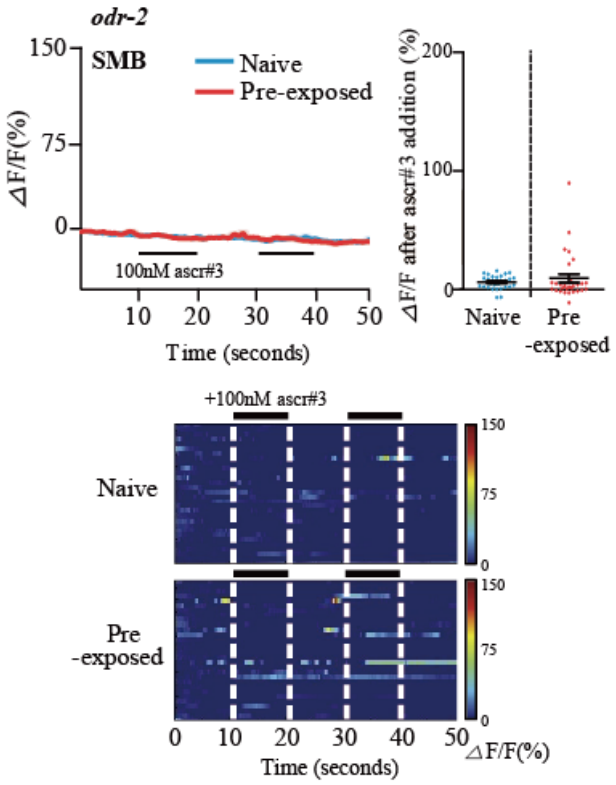

Figure 4.18 Ca^{2+} transients in SMB of animals in *odr-2* mutant

4.4 Upregulation of *odr-2* expression in SMB of ascr#3-imrprinted L1 larvae is sufficient for ascr#3 imprinting

4.4.1 GFP quantification of naive and imprinted *C. elegans*

To further investigate the role of *odr-2* in mediating increased SMB activity in of ascr#3 imprinted animals, I monitored *odr-2* expression in SMB and found that *odr-2* expression in SMB was increased after transient exposure to ascr#3 at the L1 stage and was maintained

through adulthood (Figure 4.19).

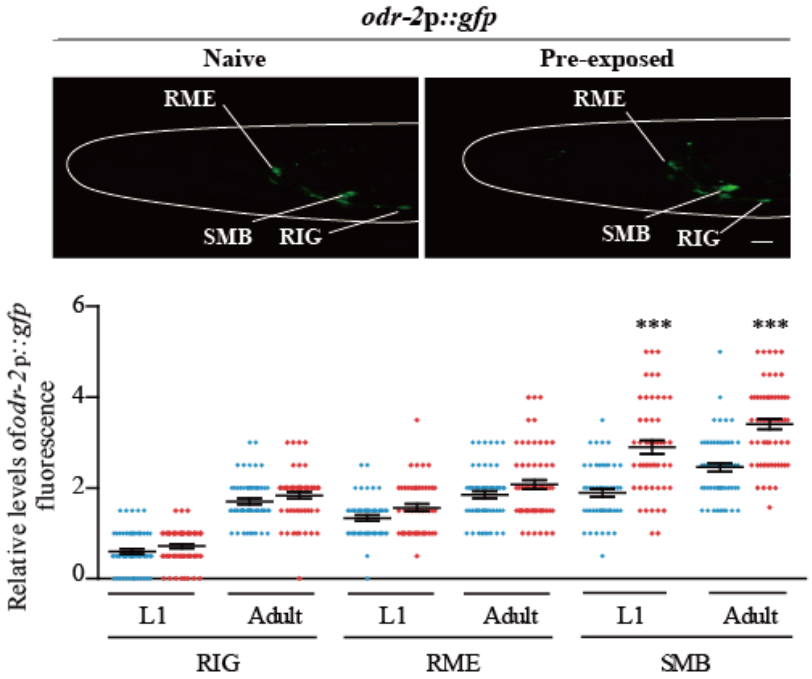

Figure 4.19 Representative images (top) and GFP quantification (bottom) of *odr-2*p*::gfp* expression in RIG, RME, and SMB of naive (shown in blue) and pre-exposed (shown in red) animals at the L1 or adult stages are shown

4.4.2 Heatshock study of non-imprinted animals

In contrast, *odr-2*p::GFP expression was unaffected in other *odr-2*-expressing cells including RIG and RME (Figure 4.19). To examine whether upregulation of *odr-2* at the L1 stage is sufficient for the increased ascr#3 avoidance in adults, I expressed *odr-2* cDNA in wild-type animals with the inducible expressed heat-shock promoter. Compared to animals with no heat-shock treated or those heat-shocked only at the adult stage, transient induction of *odr-2* gene activity at the L1 larval stage was sufficient to increase ascr#3 avoidance in adults (Figure 4.20).

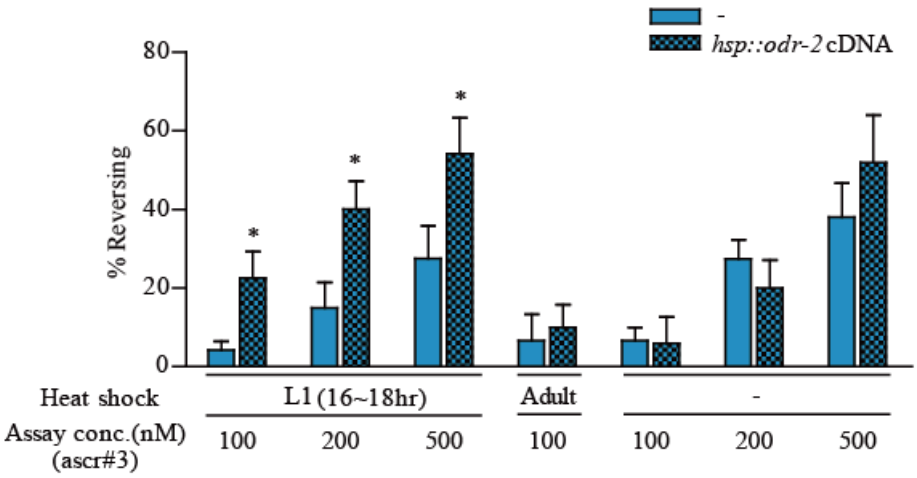

Figure 4.20 Percentage of reversal of adult transgenic animals expressing *hsp::odr-2* cDNA under heat shock at the L1 and adult stages

Thus far, our results are consistent with a model in which the increased *odr-2* expression in SMB of the ascr#3 imprinted animals causes functional changes in the ADL-SMB synapses.

4.4.3 *avr-14* study

Since the ADL neurons are glutamatergic [87], I next searched for the glutamate receptor that may mediate increased synaptic transmission from ADL to SMB in ascr#3 imprinted animals. I found that animals mutant for the *avr-14* glutamate-gated chloride channel subunit α [88] failed to exhibit ascr#3 imprinting (Figure 4.21).

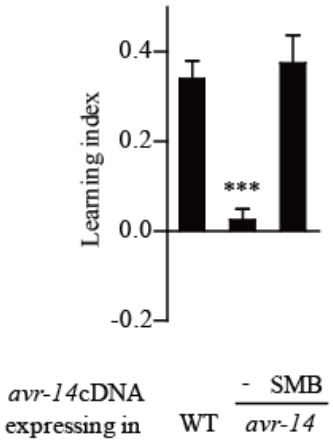

Figure 4.21 Learning index of wild-type, *avr-14* mutants, and *avr-14* mutants expressing *avr-14* cDNA under the control of the SMB specific promoter

This defect was rescued upon SMB-specific expression of *avr-14* cDNA (Figure 4.21). I also found that *avr-14* expression was significantly decreased in *odr-2* mutants (Figure 4.22).

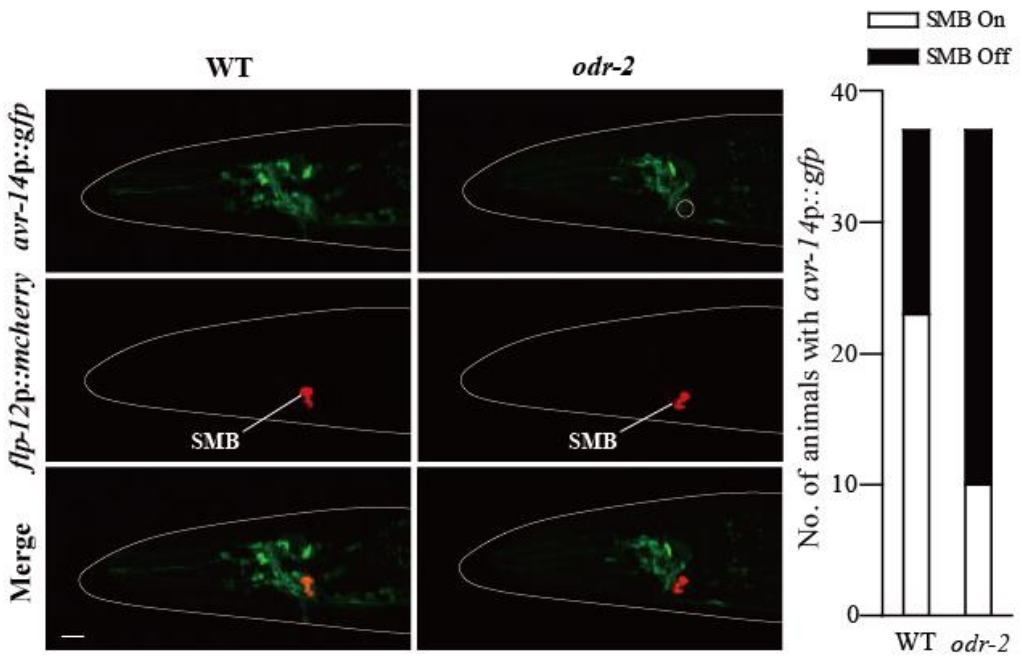

Figure 4.22 Representative images (left) and GFP quantification (right) of *avr-14* expression in SMB of WT and *odr-2* mutants

These results indicate that *odr-2* modulates the ADL-SMB synaptic transmission by regulating expression of *avr-14* in the SMB neurons.

IV. Discussion

I previously showed that the ADL sensory neurons detect *C. elegans* pheromone ascr#3 and drive ascr#3 avoidance through their chemical synapses [52]. The ADL neurons directly connect to more than twenty postsynaptic neurons, and inspection of the anatomical wiring data including the number of synapses led us to hypothesize that ascr#3 signals from ADL are transmitted to the three major post-synaptic target neurons including the AIB, AVA and AVD interneurons. In this work, I have found that under non-ascr#3 pre-exposed (naive) conditions the ascr#3 signals are mainly transmitted via the ADL-AVA synapses (Figure 4J). However, while transient exposure to ascr#3 at the L1 larval stage does not appear to affect this ADL-AVA synaptic transmission, this experience instead activates the ADL-SMB synapses which are normally inactive in naive animals, and accounts for the observed enhanced ascr#3 avoidance behavior in adult worms (Figure 4.23).

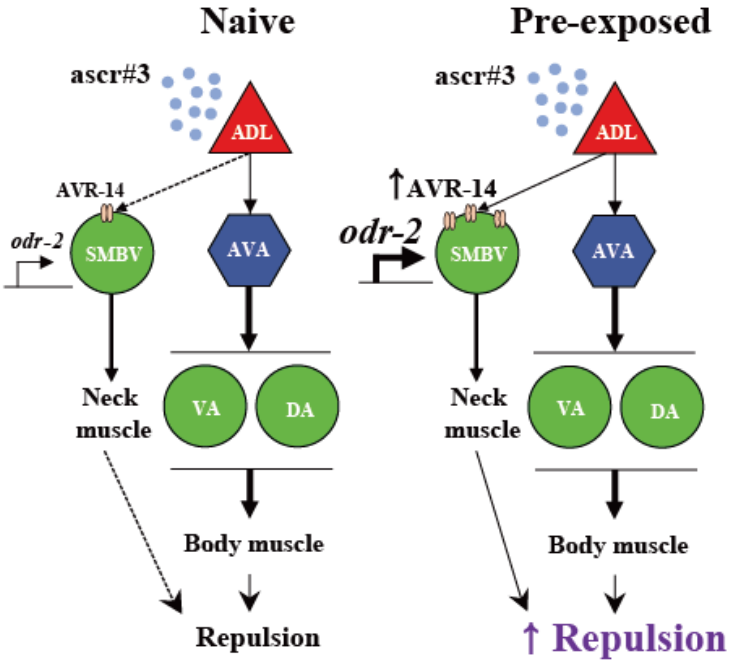

Figure 4.23 Model for circuit mechanisms underlying ascr#3 avoidance in naive or ascr#3 imprinted animals

Major neural connections of *C. elegans* nervous systems are established at the L1 stage although connectivities are subject to additional rewiring [28]; [89]; See a review by [90]. Since the long-lasting memory for ascr#3 pheromone is formed during a critical period of early development and shapes the ascr#3 avoidance behavior of adults, this learning and long-term memory process for the pheromone could be referred to as sensory imprinting [62]. *odr-2* is essential for ascr#3 imprinting. *odr-2* encodes a novel protein distantly related to murine Ly6 lymphocyte antigen protein that is tethered to membrane by a GPI (glycosylphosphatidylinositol) anchor [8]. In vertebrates, GPI anchored proteins have roles in development and neurogenesis by regulating several signaling pathways including Notch, Wnt or TGFβ [91]. It have been implicated in modulation of extracellular matrix interactions [92], cell adhesion [93], [94], signal transduction [95], regulation of plasminogen activation [96], and protection against complement-mediated lysis [97]. A mouse mutation in one Ly-6 protein causes subtle defects in T-cell activation [98]. Our results indicate that the ODR-2 GPI anchored protein regulates expression of the AVR-14 glutamate-gated chloride channel which leads to changes in synaptic activity between ADL and SMB. Since ODR-2 proteins are localized on cell bodies and neuronal processes [99], it is likely to mediate changes in gene expression indirectly, and it could be intriguing to investigate signaling pathways by which *odr-2* regulates gene expression. Recently, it has been proposed that the long-term memory formation may be mediated epigenetic mechanisms [100]. More specifically, neuronal activation could lead to chromatin remodeling of target genes, resulting in stable changes in gene expression. Thus, studies on epigenetic regulation of *odr-2* expression would be the next step to understand molecular mechanisms underlying ascr#3 imprinting.

Understanding how individual synapses are functionally or anatomically altered upon

sensory imprinting is the essential first steps towards being able to dissect molecular and neuronal mechanisms underlying sensory imprinting and other forms of behavioral plasticity. Given that structures and functions of neural circuits are evolutionarily conserved, I expect that our work will lead to a general framework for understanding how circuits are modulated in higher animals including humans.

V. Conclusion

The conclusion that the SMB neurons are the critical site for mediating ascr#3 imprinting is supported by four findings: 1) *lim-4* mutants in which SMB functions are abolished fail to imprint ascr#3 experience; 2) The SMB neurons respond to ascr#3 exposure in ascr#3-imprinted but not in naive animals; 3) Expression of *odr-2* that is necessary and partly sufficient for ascr#3 imprinting is increased specifically in SMB of ascr#3-imprinted animals; 4) The *odr-2*-regulated *avr-14* gene also acts in SMB to mediate ascr#3 imprinting. Laser ablation of the SMB motor neurons causes increased reversal frequency and wave amplitude of forward locomotion [85]; [49]. Since the SMB neurons innervate head and neck muscles, and ascr#3 exposure now activates the SMB neurons in ascr#3 imprinted animals, our results suggest that early ascr#3 experience causes the SMB neurons sensitize to subsequent ascr#3 exposure likely via altered glutamatergic signaling and modulate backward movement via the alteration of head/neck muscle activity (Figure 4.23).

An essential step of neural development is neural circuit formation in which neurons precisely identify and make connections with their synaptic partners. This process appears to be highly plastic and therefore can be affected by the external environments or internal physiological conditions. Although electron micrograph reconstruction studies identified synapses

between the ADL sensory neurons and the SMB neurons [28], our data indicate that these synapses do not transmit ascr#3 signals from ADL to SMB in naive animals, indicating that these synapses are not functional or synaptic transmission via these synapses is not sufficient to excite SMB. However, SMB of ascr#3 imprinted animals showed strong and consistent Ca^{2+} transients upon ascr#3 exposure, suggesting that ADL-SMB synapses are changed to be functional. These results prompt us to investigate whether quality or quantity of ADL-SMB synapses are changed in ascr#3 imprinted animals.

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요 약 문

페로몬 감각 각인의 기초가 되는 회로 메커니즘연구

본 논문은 전체 게놈이 최초로 알려졌으며 유일무이한 개체수준의 뉴로커넥톰이 구축된 예쁜꼬마선충을 모델시스템으로 연구를 진행하였다. 이전 연구들은 각 신경들의 구조와 기능에 대한 미시적 수준에 머물러, 뉴로커넥톰의 역할과 기능에 관한 거시적 수준에서의 연구는 미약한 실정이었다. 본 논문은 세계최초로 페로몬에 대한 '각인' 이라는 장기기억 현상을 발견하였다. 먼저 이러한 행동가소성을 야기하는 원인 유전자를 찾아 신경세포 수준에서의 접근법을 모색하였고, 그 후 분자생물학 및 유전학적 방법을 기반으로 관련 신경회로의 작동원리를 규명하여 스마트 뉴로커넥톰을 구축하는데 일조하였다. 따라서 본 논문은 뉴로커넥톰의 작동원리 규명, 향후 뇌신경계 기능 구현, 다양한 신경정신 질환의 원인 규명 및 치료로의 활용에 획기적 단서를 제공할 것이다.

핵심어: 뉴로커넥톰, 각인, 시냅스