Habituation in Caenorhabditis elegans : Its analysis by using behavioral,neural circuit and genetic approaches

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学 位 論 文 要 旨

ABSTRACT

We studied on habituation in the nematode *Caenorhabditis elegans* by using behavioral, neural circuit, and genetic approaches. Worms in the habituated states evoked by one site touch are still sensitive to touch on the rest sites. To identify neuron that participates in habituation, we ablated systematically neurons constituting the neural network for the mechanical stimuli with a laser microbeam and investigated the resulting habituation of the operated animals. We could not find any abnormality on habituation of animal defective in each mechanosensory neuron or interneuron except the AVD interneurons. The AVD interneuron plays a critical role on the habituation in intact animal. In order to identify genes that are responsible to the tap-related habituation in *C. elegans* and we adopted cDNA microarrays. We isolated mRNA as probe from L1 larvae with or without tap stimuli. We compared the hybridization density in microarrays with the mRNAs as probes and found 248 genes that are up or down regulated during the tap stimulation. The cells

expressing those identified genes have been studied by in situ hybridization. We found 8 genes expressed at the specific region in the nervous system. Finally, we identified several genes contributing to the habituation by using double-stranded RNA interference.

INTRODUCTION

Learning behavior is classified into associative and nonassociative learning. For associative learning, animals are exposed to different stimuli to learn the relationship of one stimulus to another stimulus or the relationship of a stimulus to behavior. For nonassociative learning, animals are exposed once or repeatedly to a single type of stimulus and learn about the properties of the stimulus. So, habituation is the foundation of selective attention. Habituation allows animals to ignore common, irrelevant stimuli so that they can attend to stimuli important for survival. Habituation has been demonstrated in many organisms tested including single celled protozoa, *C. elegans*, *Drosophila*, *Aplysia*, fish, rat, and human. For example, in the gill-withdrawal reflex of *Aplysia*, changes in synaptic strength with habituation were observed in the connections between sensory neurons and motorneurons. However little is known about the intrinsic and synaptic properties of neurons, including molecular mechanisms underlying behavioral modifications. The nervous system of *C. elegans* is very simple, and its complete morphology, cell lineage and genome sequences are already known.

We are trying to understand on habituation in *C. elegans* by using behavioral, neural circuit, and genetic approaches.

RESULTS AND DISCUSSION

C. elegans moves backward when subjected to a touch stimulus. This response shows habituation by repeated touch stimuli. We tested interrelationships of body touch, touch on the nose, touch on the head, and touch on the tail, all of which stimulate specific mechanosensory neurons. Animals in the habituated states evoked by one site touch are still sensitive to touch on the rest sites. If the plasticity occurred at the level of the integral processing phase: final interneurons and/or motorneurons, reversal response to touch on the rest sites that use the same final interneurons and motorneurons should have been affected by habituated states evoked by one site touch. Because behaviors were not affected by the habituation induced by other touch sites the most likely sites of plasticity for habituation to touch are the parallel processing level: mechanosensory neurons and/or initial stages of interneurons (Fig. 1).

Withdrawal responses elicited by head touch are controlled by

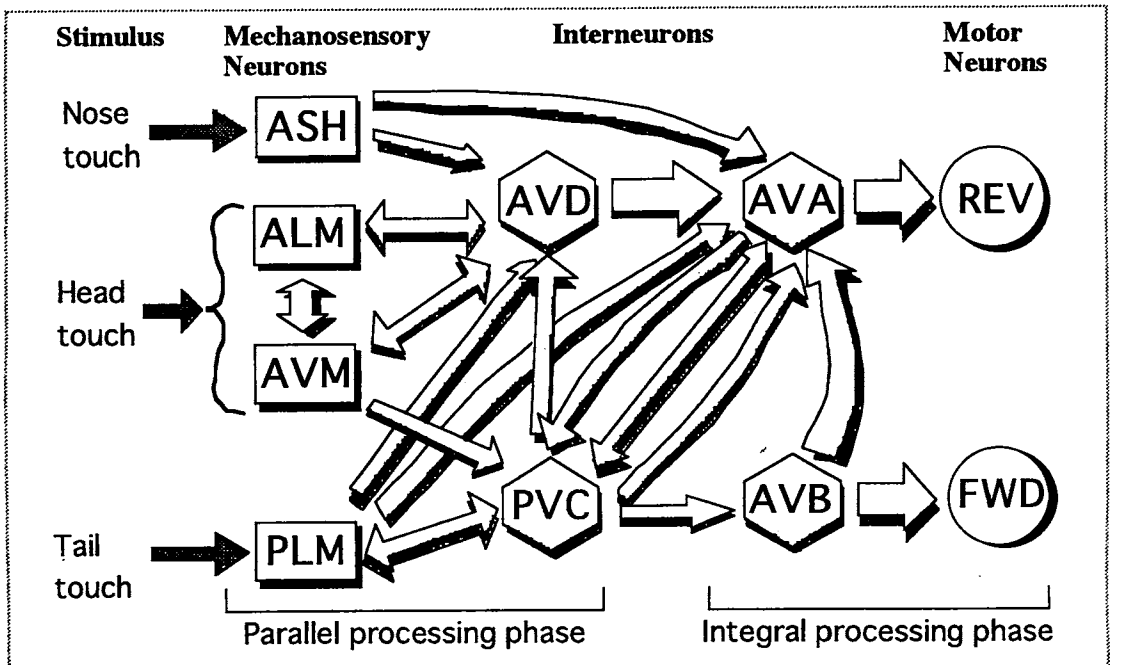

Figure 4 Signal flow in the touch-reflex circuit

anterior mechanosensory neurons (AVM and a pair of ALM), by four pairs of interneurons (AVA, AVB, AVD, and PVC) and by motorneurons (Fig. 2). To identify neuron that participates in habituation, we ablated systematically these neurons constituting the neural network with a laser microbeam and investigated the resulting habituation of the operated animals (Table 1). Ablation of single-class cell caused no abnormality on habituation of animals

Table 1. Summary of the habituatios of animals lacking the neurons of the touch circuitry

Neurons killed	Number of animals tested	Initial response magnitude[a]	Number of stimuli for habituation[b]
None	25	137	21
Mechanosensory neuron			
ALMLR$^-$	20	-	-
AVM$^-$	20	90	23
PLMLR$^-$	20	103	19
Interneuron			
AVDLR$^-$	20	88	11
AVALR$^-$	24	64	24
PVCLR$^-$	23	102	23
AVBLR$^-$	22	89	21
Combination			
AVALR$^-$/PVCLR$^-$	21	50	23
AVBLR$^-$/PVCLR$^-$	20	78	19
AVBLR$^-$/AVDLR$^-$	22	63	15
PVCLR$^-$/AVDLR$^-$	23	67	23
ALMR$^-$/AVM$^-$	25	90	9
ALMR$^-$/AVM$^-$/AVDLR$^-$	23	81	9
ALML$^-$/AVM$^-$/PVCR$^-$	20	83	22

Contribution to the habituation was evaluated by ablating each class of neurons that constitute the network for mechanical stimulus. Ablations of AVDLR, AVBLR/AVDLR, ALMR/AVM and ALMR/AVM/AVDLR cause rapid habtuation.
a; the magnitude of response to the first touch stimulation is expressed as a percentage of the individual's body length.
b; the number of stimulations for the population being habituated are indicated.

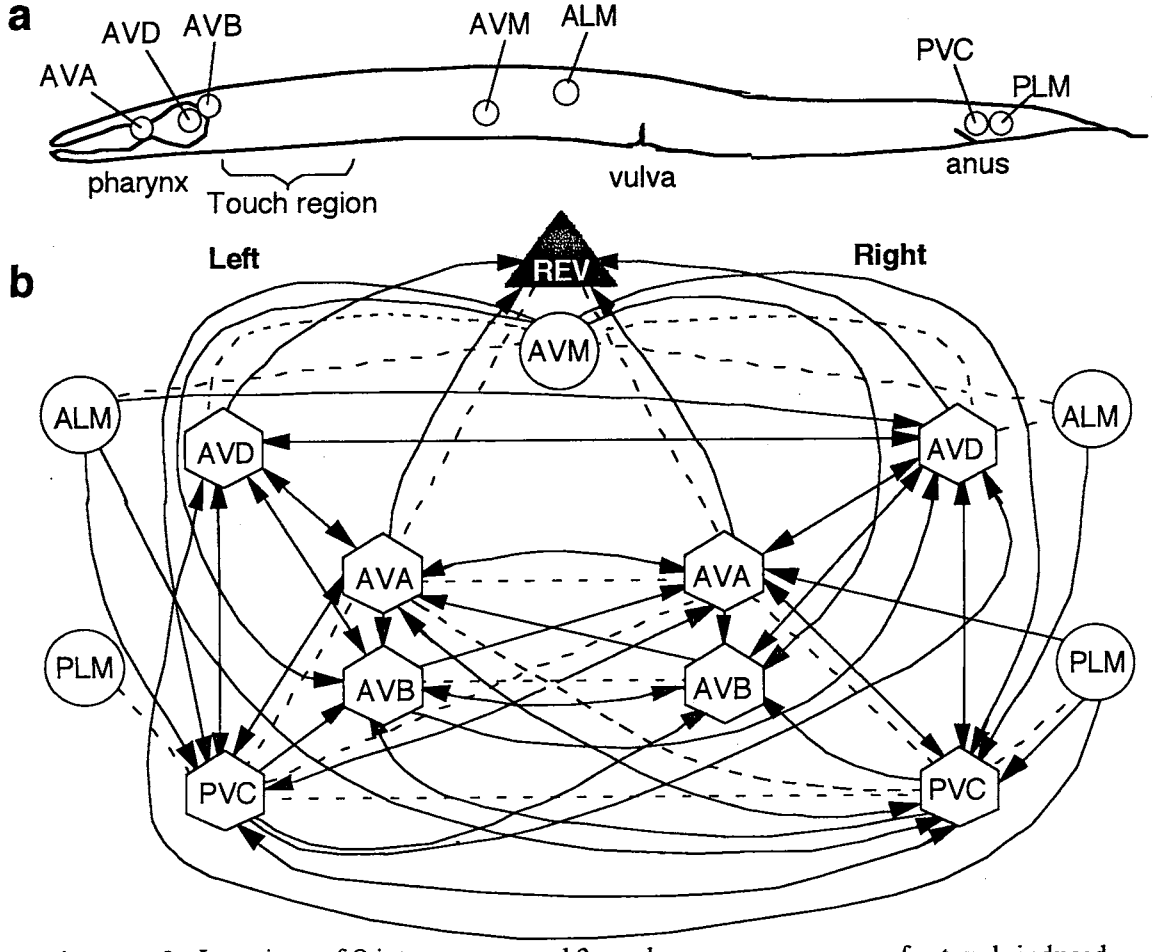

Figure 2 Locations of 8 interneurons and 3 mechanosensory neurons for touch-induced backward movement (a) and a simplified schematic representation of the touch circuitry with synaptic relationships (b). The arrows and dotted lines represent chemical synapses and gap junctions, respectively. The diagrams are modified from Chalfie et al., (1985) and Wicks et al., (1996).

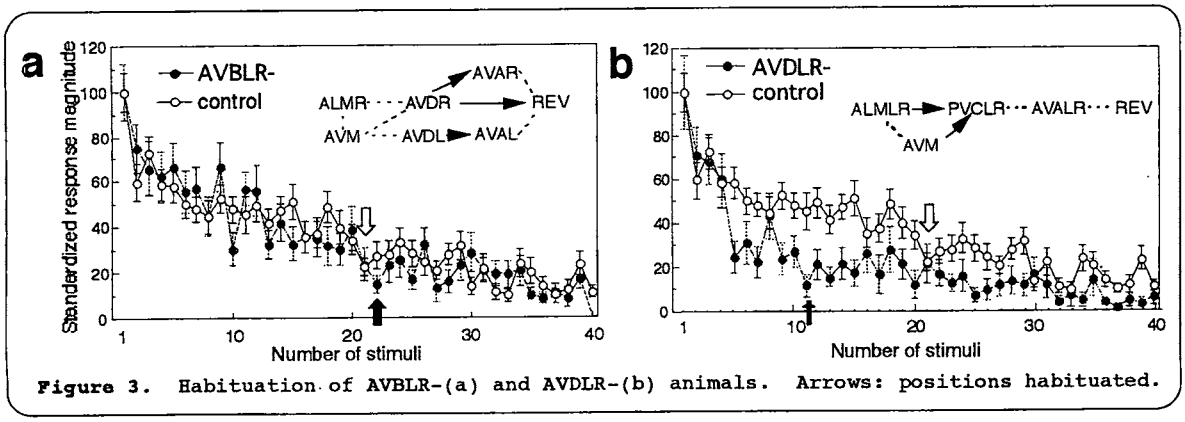

Figure 3. Habituation of AVBLR-(a) and AVDLR-(b) animals. Arrows: positions habituated.

except of animals ablated the bilateral AVD interneurons (AVDLR) (Fig. 3). Animals lacking AVDLR interneurons ($AVDLR^-$) were habituated more rapidly than intact

animals. In cross-class cell kill, rapid habituation was found in AVBLR$^-$/AVDLR$^-$, ALMR$^-$/AVM$^-$, and ALMR$^-$/AVM$^-$/AVDLR$^-$. A simplified schematic neural pathway for the head touch withdrawal response is shown in figure 4 (a). We postulated that, in habituated state, signals are transmitted via the gap junctions without attenuation, but the signals via the chemical synapses rapidly decrease. In intact animals, after repeated stimuli, head touch stimulus received by ALMLR and AVM mechanosensory neurons
must be transmitted to AVDR via gap junctions, and then transmitted to AVAR and motorneurons via chemical synapses in which synaptic changes occurred (Fig. 4(b)). While in the rapid habituated animals caused by ablation, the stimulation received at the ALML mechanosensory neuron must be transmitted to PVCL through chemical synapses, and then transmitted to AVDR and motorneurons via gap junctions. In these animals
the most likely sites of plasticity for habituation are the ALML chemical synapses onto the PVCL interneuron (Fig. 4(c)).

To understand the molecular basis of habituation and retention of habituated state, it is essential to identify genes individually

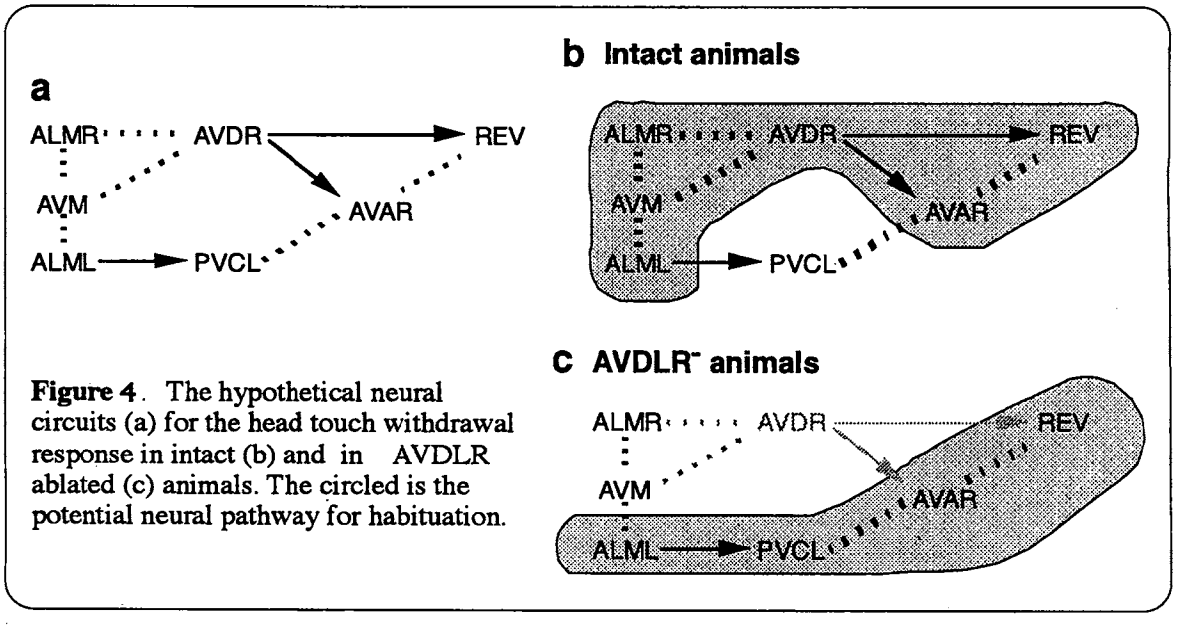

Figure 4. The hypothetical neural circuits (a) for the head touch withdrawal response in intact (b) and in AVDLR ablated (c) animals. The circled is the potential neural pathway for habituation.

or systematically. The development of microarray systems for gene expression profiling permits systematic screening of a large number of genes involved in biological processes. Therefore, we used a microarray consisting of 4,899 cDNAs of *C. elegans* to detect alterations in gene expression in the process of habituation. We isolated mRNA as probe from L1 larvae with or without training of 180 times 30-s interstimulus tap stimuli. We compared the hybridization density in microarrays with the cDNAs as probes between trained and control animals. We found 2.0-fold or greater increases in the expression of 143 genes and 2.0-fold or greater decreases in the expression of 105 genes at 1.5 hours after tap stimuli. The up and down regulated genes are likely to be involved in not only chromosome dynamics, signal transductions, transcription factors, transporters, and ion channels but also metabolisms, mitocondria. However many genes are still functionally unknown. The cells expressing those identified genes have been studied by *in situ* hybridization.

We found 8 genes are expressed in the nervous system in the head region, including C03F11.9, ZK1236.2, T11B7.4a, B0507.1, C05C12.3, C49G7.4, C24A8.4, and *unc-52* (Table 2). To examine their function in *C. elegans*, we performed genetic interference mediated by double-stranded RNA (dsRNAi) using bacteria to deliver dsRNA. We

Table 2 List of 8 genes expressed in the nervous system

Up-regurated genes			
Clones	genes	Description	Functional clsses
yk544c6	C03F11.9	unknown function	
yk116d10	ZK1236.2	Nucleolin, Chromatin organization modifier	Chromosome dynamics
Down-regurated genes			
Clones	genes	Description	Functional clsses
yk386d6	T11B7.4a	LIM domains	Transcription factor
yk373f8	B0507.1	EGF-like domains	Signal transduction
yk308e9	C05C12.3	Calcium channels TM region	Cation channel
yk272h9	C49G7.4	unknown function	
yk44g5	C24A8.4	G-protein coupled receptor	Signal transduction
yk554e12	unc-52	Laminin B domain	Cell structure

fed engineered bacteria expressing dsRNA to *C. elegans* and investigated the resulting habituation of those animals. Animals continuously exposed to dsRNA of C05C12.3 and T11B7.4a genes were rapidly habituated more than control animals (Fig.5). Furthermore, dsRNAi of the C05C12.3 gene resulted in retention of habituation with slow recovery from habituated state. The C05C12.3 gene encoding the transmembrane region of the voltage-gated calcium channel. A dsRNAi of the C05C12.3 gene might modulate the presynaptic Ca2+ influx that triggers release of neurotransmitters. Habituation is the foundation of selective attention. Then, down regulation of C05C12.3 gene expression might allow animals to ignore redundant and irrelevant mechanical stimuli, so that they can attend to other stimuli important for survival.

In summary we studied the habituation behavior with animals ablated neurons constituting the touch-reflex circuit. Using microarray technique we identified numerous potential genes contributing to the habituation. By RNAi experiments with these genes, we found potential genes affecting on the habituation behavior.

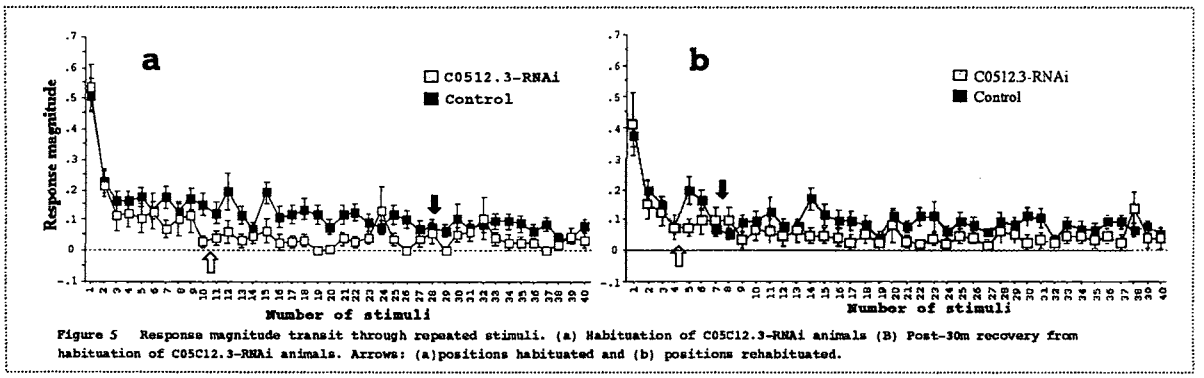

Figure 5 Response magnitude transit through repeated stimuli. (a) Habituation of C05C12.3-RNAi animals (B) Post-30m recovery from habituation of C05C12.3-RNAi animals. Arrows: (a)positions habituated and (b) positions rehabituated.

学位論文審査結果の要旨

神経高次機能，特に学習に伴う記憶獲得機構解明は生命科学で最重要課題の一つである。本研究ではセンチュウ *C. elegans* を用い非連合学習の一種である慣れ学習に働く神経回路網と寄与する遺伝子について調べた。

C. elegans に弱い機械的刺激を与えると後退反応をする。くりかえし与えるとやがて反応しなくなる慣れ現象が観察される。本研究ではまずこの学習行動の解析系を確立した。確立された解析系を用いタッチ刺激を与えた際の慣れ現象に働く神経について調べた。タッチ刺激に働く神経回路網は5感覚ニューロンと8介在ニューロンから構成されている。これらのニューロンをレーザー顕微破壊で焼却しても学習行動に影響しなかった。しかし，AVDL, AVDR 介在ニューロンあるいは AVM, ALMR 感覚ニューロンを同時に破壊すると慣れ行動に変化が生じた。このことは本慣れ学習にはタッチ回路網全体が必要ではなく特定神経回路が重要な働きをしていることを示している。

本研究では次いで本慣れ学習に働く遺伝子について調べた。そのため，学習させた *C. elegans* から mRNA を単離し，cDNA microarray を用いハイブリダイゼーションし，学習させない *C. elegans* からの mRNA との比較から発現が変化する遺伝子同定を行った。その結果 248 遺伝子を同定した。これらの遺伝子群から特に神経系での発現に違いが観察された 8 遺伝子の本学習への寄与を RNAi 法で研究した。

以上により，本研究は学習に働く特定神経細胞を明らかにしたばかりでなく遺伝学的手法で記憶獲得機構解明の道を拓いた。よって本論文は博士（理学）論文に値すると判定された。