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研究課題名（和文）Identification of neuron-glia signaling mechanisms required for synaptic plasticity and memory formation

研究課題名（英文）Identification of neuron-glia signaling mechanisms required for synaptic plasticity and memory formation

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研究成果の概要（和文）：ヒトは年を取ると記憶力が減退する（加齢性記憶障害）。我々はショウジョウバエを用いて、長期記憶の加齢性記憶障害の原因解明を試みた。先ず、通常の長期記憶の形成には、学習後の蛋白合成を伴って短期記憶が長期記憶に転換される期間中に、ドーパミン性ニューロンの活性が低下することが必要なことを見出した。更に、ドーパミン性ニューロンの活性阻害による長期記憶の増強は、若いハエに比べ老化したハエにおいて著しいことを明らかにした。また、ドーパミン性ニューロンの内、長期記憶の加齢性記憶障害に関わるサブクラスを同定し、このニューロン活性が加齢によって増進することを示す予備的実験結果を得た。

研究成果の概要（英文）：As we age, we suffer a decrease in memory, known as age-related memory impairment or AMI. Here we examined the caused of age-related impairments in long-term memory (LTM) in Drosophila. We found that normal LTM formation requires decreased neuronal activity during memory consolidation, a period of time after training during which protein synthesis occurs and short forms of memory are converted or consolidated into LTM. In particular, we found that inhibiting activity of dopaminergic neurons improves memory consolidation. Further, inhibiting activity of dopaminergic neurons improved LTM more in old flies compared to young flies. This indicated that one cause of age-related impairments in LTM is increased dopaminergic activity during consolidation in old flies. We identified a subset of dopaminergic neurons responsible for age-related impairments in LTM, and have evidence showing that activity of these neurons increases with age.

研究分野：　Neurobiology of learning and memory

キーワード：　Drosophila　Learning and Memory　glia

１．研究開始当初の背景

As we age, we suffer a decrease in memory. This phenomenon is known as age-related memory impairment (AMI). AMI is a serious medical and social issue, but its causes and mechanisms of action are still relatively unknown. In order to understand AMI, we need to understand how memory is formed, stored, and recalled in young brains, and determine how these processes are affected upon aging. *Drosophila* are a useful model organism in which to study these effects.

During aversive olfactory learning in *Drosophila*, flies are exposed simultaneously to an odor and aversive stimuli such as electrical shocks. Flies learn to associate the odor with pain and subsequently avoid the odor. In the fly brain, these associations are formed in structures known as the mushroom bodies (MBs). Odor information is transmitted to the MBs through cholinergic inputs from the antennae via the antennal lobes. Individual odors activate different sparse subsets of MB neurons such that odor identity can be encoded by the specific subset of activated MB neurons. MB output neurons connect MB neurons to other brain areas, and activation of different subsets of MB output neurons cause flies to exhibit avoidance or approach behaviors. Thus current models propose that learning consists of plasticity between odor encoding MB neurons and specific MB output neurons. Electric shock information is thought to be transmitted to the MBs via specific dopaminergic neurons, and simultaneous activation of shock-transmitting dopaminergic neurons and odor-encoding MB neurons is thought to strengthen connections between the odor-encoding MB neurons and specific MB output neurons that induce avoidance behaviors.

Previously, we determined that aging affects two specific types of memory in *Drosophila*, a short-lasting form of memory known as middle-term memory and a long lasting form of memory known as long-term memory. We found that impairments in middle-term memory were caused by age-related defects in glial production of a neuromodulator, D-serine. D-serine is a coactivator of NMDA-type glutamate receptors, suggesting that defects in middle-term memory are caused by reduction of glutamate activity in old flies.

２．研究の目的

The overall goal of this grant was to identify molecular mechanisms causing age-related impairments in long-term memory, using *Drosophila* as a model organism.

In order to achieve this goal, we needed to:

1) Identify the function of glial cells in long-term memory formation.
2) Identify the role of dopaminergic neurons in long-term memory formation.
3) Determine how glial activity and subsequent dopaminergic activity changes during aging to cause age-related memory impairments.
4) Update current models to obtain a more accurate model for how associative memories are formed in the MBs.

３．研究の方法

Learning and memory assays

During one training session, flies were exposed simultaneously to an odor and electrical shocks for one minute in an apparatus known as a teaching machine. They were subsequently exposed to a second odor in the absence of electrical shocks. Flies learned to associate the shock-paired odor with pain, and the second odor with safety. Learning and memory of this association was measured at various time points after training by allowing the flies to choose between the two odors in a T-maze and quantifying how much the flies preferred the non-shocked odor to the shock-paired odor.

Spaced training, which produces long-term memory, consisted of ten training sessions with 15 minute rest intervals between each training. Massed training, which produces anesthesia-resistant memory, consisted of ten training sessions without rest intervals.

Drosophila strains

To inhibit neuronal activity, we used flies expressing a temperature sensitive *shibire* transgene, which inhibits neurotransmitter release at restrictive temperatures. To artificially stimulate neuronal activity, we used flies expressing a *trpA1* transgene, which activates neurons at high temperatures. Both temperature sensitive *shibire* and *trpA1* were expressed in specific dopaminergic subpopulations using driver lines obtained from *Drosophila* stock centers.

Immunohistochemistry

Neuron numbers and morphology were analyzed by expressing GFP in neurons using specific drivers. Antibodies to GFP were used to visualize neurons in fixed

dissected brains using confocal microscopy.

In vivo Ca^{2+} imaging
The fluorescent Ca^{2+} sensor, GCaMP3 was expressed in specific dopaminergic neurons using specific drivers. Neuronal activity was measured in these neurons in naïve and spaced trained, young and old flies by dissecting off the cuticle covering the brain in living flies, and observing Ca^{2+} activity using confocal fluorescence microscopy.

４．研究成果

1) *Characterization of the role of glia in age-related memory impairment in Drosophila*
We characterized the effects of aging on long lasting consolidated forms of memory, and determined that aging affects one type of memory known as long-term memory (LTM), but not another type, known as anesthesia-resistant memory (ARM). When we characterized LTM further, we determined that inhibiting activity of dopaminergic neurons during consolidation improves memory in both young and old flies. This suggested that dopaminergic activity either inhibits consolidation or accelerates forgetting. Importantly, we found that inhibiting dopaminergic activity improved LTM to equivalent levels in young and old flies. In other words, age-related impairments in LTM are observed in normal flies, but not in flies where dopamine activity is inhibited during consolidation. This suggested that age-related impairments in LTM were caused by an increase in dopaminergic activity during consolidation at old ages. Collaborating the Motomi Matsuno at the Tokyo Metropolitan Institute of Medical Science, we identified a subset of dopaminergic neurons that is responsible for inhibiting consolidation and accelerating forgetting. We determined that these cells do not seem to change morphologically during aging; they do not die during old ages, and their axonal and dendritic processes do not show gross morphological changes. However, we found evidence of increased activity of these neurons during memory consolidation in old flies compared to consolidation in young flies. This suggests that inhibition of these neurons is required for normal consolidation. Old flies are unable to inhibit this activity leading to defects in LTM. Again, in collaboration with Motomi Matsuno, we found that inhibition of AMI-associated dopaminergic neurons depends on glial cells. Glial expression of a glutamate transporter inhibits glutamate activation of these cells. Old glial cells are unable to increase expression of this glutamate transporter leading to dysfunction of LTM consolidation.
Incorporating our current findings with results from our previous studies, we propose a model where glutamate activity needs to be high during memory formation, and low during memory consolidation. Glial cells support both high and low glutamate activity by producing the neuromodulator D-serine to increase glutamate signaling during formation, and by increasing expression of a glutamate transporter, which inhibits glutamate signaling, during consolidation. Age-related impairments in both short-lasting memory occur because old glial cells are unable to produce sufficient amounts of D-serine, while age-related impairments in LTM occur because old glial cells are unable to produce sufficient amounts of glutamate transporter.

2) *Development of an updated model for associative learning in Drosophila*
Current models propose that electrical shock information is conveyed to the MBs via dopaminergic activity, and this activity induces plastic changes between MB neurons and MB output neurons to alter behavioral responses to odors. However, it is unlikely that electrical shocks on their own induce behavioral changes. Instead, based on mammalian models, learning and behavioral changes should occur based on the differences between what an animal expects, and what actually happens. In other words, an animal will learn if it experiences an electrical shock when it was not expecting one, but not if it experiences a shock that it was already expecting. This is known as prediction error theory. In order to incorporate prediction error into current models of *Drosophila* learning, we propose a novel model where two different types of memory are stored in the MBs. One is called the semantic memory and consists of what the animal predicts will occur upon exposure to an odor based on previous experiences. The second is called the episodic memory and consists of what the animal actually experiences during the latest exposure to the odor. Dopaminergic neurons calculate the differences between semantic and episodic memories to update the semantic memory and update behavioral responses. We are currently preparing this new theory for publication.

５．主な発表論文等

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６．研究組織

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