Molecular Correlates of Training that Contribute to Memory Formation after Learning that Food is Inedible in *Aplysia*

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There is still much more to be understood about the mechanisms governing the formation of memories. The marine gastropod opisthobranch mollusk, *Aplysia*, provides a model for studying learning due to its ability to form long-term and short-term memories in response to a stimulus and its relatively large neurons. In addition, their neural circuitry is made up of less neurons and is relatively simple in comparison to the mammalian brain, all of which make it an effective model to study memory. The molecular correlates of learning in *Aplysia*, are a topic of interest and we are investigating them to better understand the molecular mechanisms involved in memory formation. It is well established in the scientific literature on memory that certain transcription factors are associated with memory formation such as C/EBP and CREB1. In *Aplysia*, CREB1 is further processed to produce CREB1α and CREB1β. These transcription factors turn on genes that will produce mRNA to be transported from the nucleus to the synapses to produce long-term changes that occur later during memory consolidation. Therefore, we looked at the changes in C/EBP, CREB1α, and CREB1β expression in *Aplysia* to better understand the molecular correlates of learning.

Background

Learning in *Aplysia*

In response to an associative learning training task, animals form memories that a specific food is inedible.

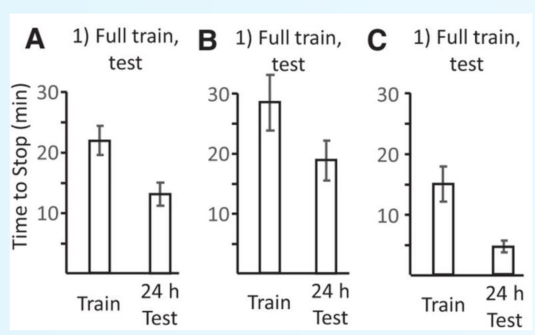

Figure 1. Animals form memories after receiving 15 minutes of training. Training included 15 minutes of introducing the animals to an inedible food. All three groups formed significant memory seen in the decrease in time to stop feeding behaviors 24 hours after the training.

- This shows that training with inedible food provides a way to stimulate memory formation in *Aplysia*.

Molecular Correlates

CEBP expression increases in the sensory cells of the buccal ganglia after training, but not lip stimulation.

- C/EBP alone may not be enough to form memories, but it may still contribute to memory formation along with other molecular correlates.

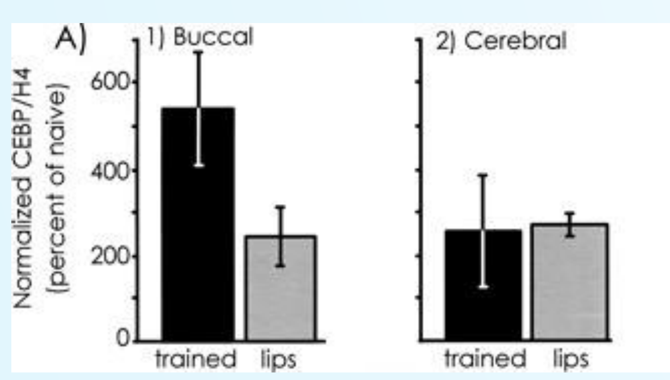

Figure 2. Lip stimulation does not produce a significant increase in the expression of C/EBP in the buccal or cerebral ganglia. Training for 15 mins produces a significant increase in the expression of C/EBP in the buccal ganglia, not the cerebral ganglia.

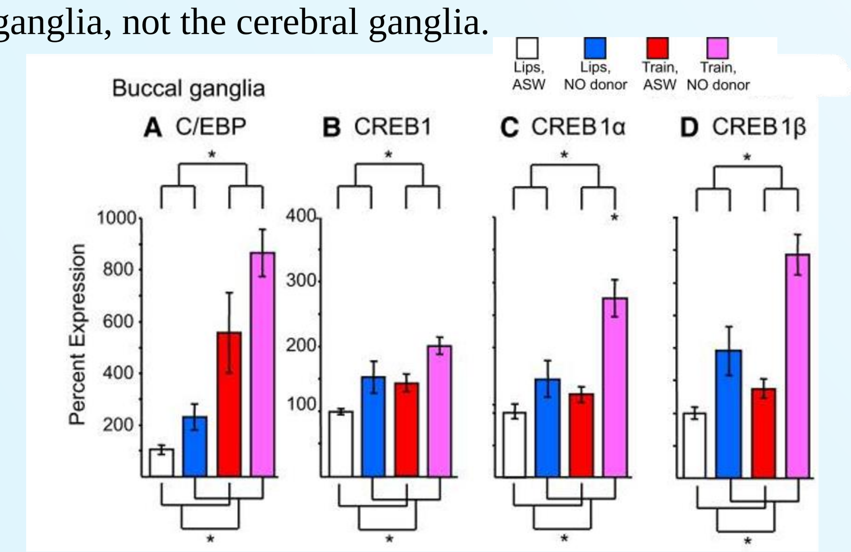

Figure 3. Changes in gene expression of the buccal ganglia in response to training. Expression levels were measured 2 hrs after treatment. The levels of mRNA expression were expressed as a percentage of the mean value of animals that had been treated with both lip stimulation and saline. (*C*) There is a significant interaction between the treatment with Nitric Oxide and training that produces an increase in CREB1α expression. Significant increases in gene expression of both buccal and cerebral ganglia were found when animals underwent treatment, except C/EBP.

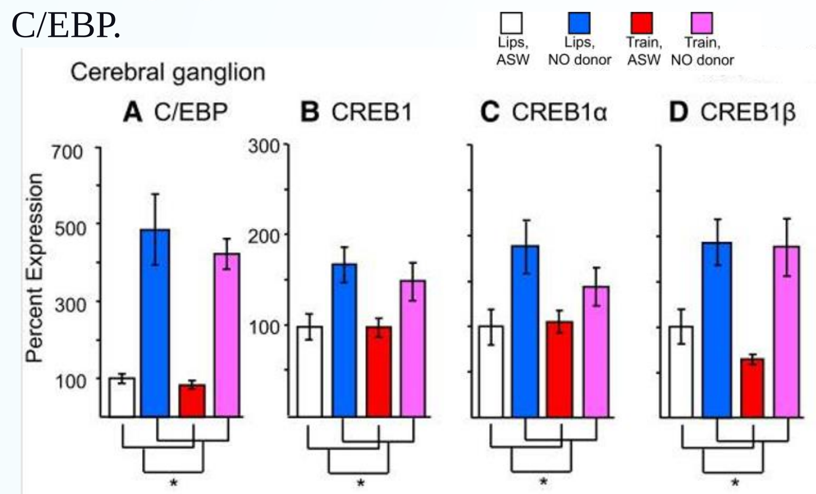

Figure 4. Changes in gene expression of the cerebral ganglia in response to training. Increases in expression of most genes, even in response to treatments that did not cause memory formation. C/EBP remains the exception with only a significant increase during training.

Methodology

- Groups of five and six received a treatment of either fifteen minutes of lip stimulation or full training.

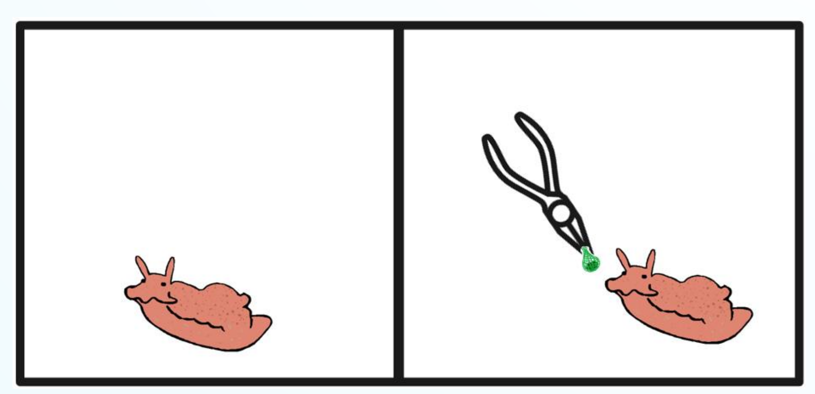

Figure 5. Training and Lip Stimulation Set up. Feeding responses were induced in the animals through an associative learning task that included (a) lip stimulation with a specific food; (b) attempts to swallow the food; (c) failure of the food to enter the gut. Lip stimulation differed in that the food was removed before the animal bit down. Each tank had two *Aplysia* separated by a barrier.

- The buccal and cerebral ganglia of the trained and naive groups were excised both 15 mins and 2 hrs after training.
- Five experimental groups: Short train, Long train, Short Lip, Long Lip, and naïve.
- Total RNA was extracted from the cerebral and buccal ganglia and then RNA concentration was measured using a spectrophotometer. This was followed by cDNA synthesis and quantification of gene expression by quantitative polymerase chain reaction (qPCR). The mRNA levels were then analyzed utilizing the comparative Ct method.

Preliminary Results: CREB1α Buccal

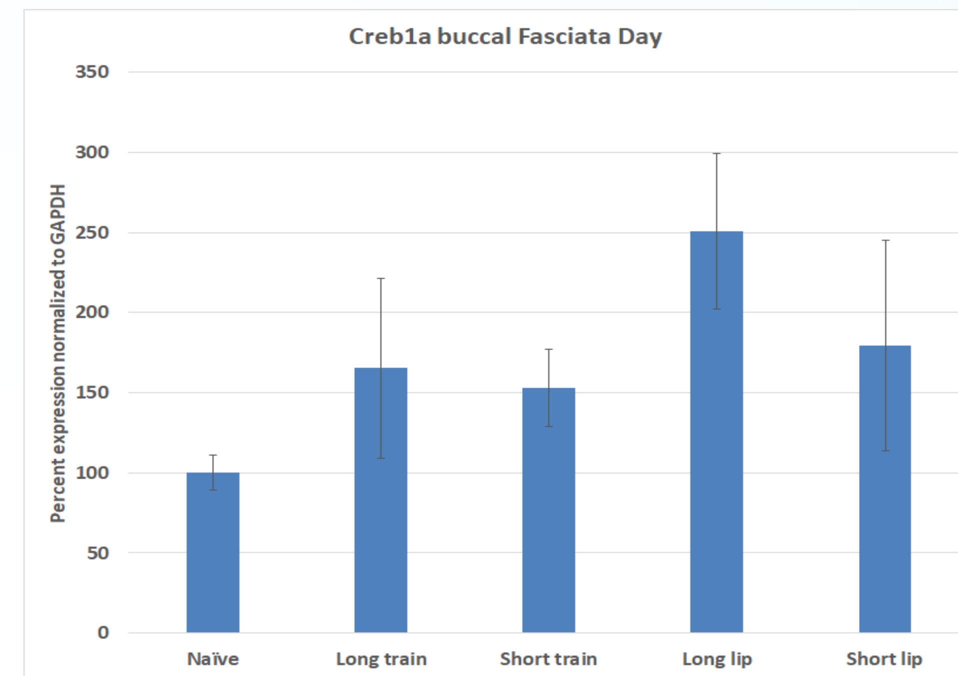

Figure 6. CREB1α mRNA expression levels in the buccal ganglia. The level of mRNA expression was expressed as a percentage of the mean value of the naïve animals. There is no significant difference seen between the lip stimulation and full training. Overall, the naïve group has lower CREB1α expression than the treated groups.

Preliminary Results: CREB1α Cerebral

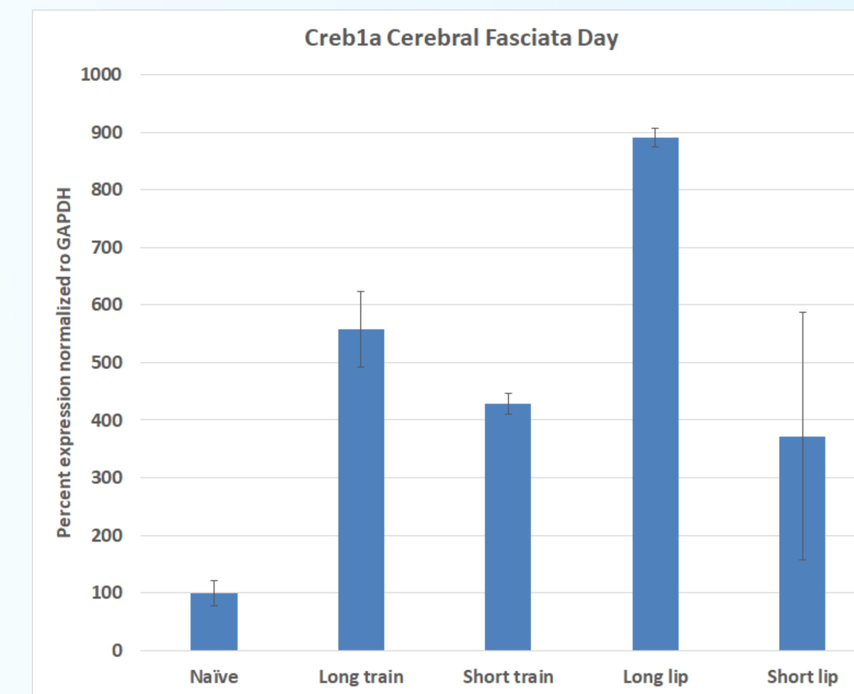

Figure 7. CREB1α mRNA expression levels in the cerebral ganglia. The level of mRNA expression was expressed as a percentage of the mean value of the naïve animals. In the short-trained groups, there was no significant difference seen between the lip stimulation and full training. The naïve group has lower CREB1α expression than the treated groups.

Primers Used for qPCR

GAPDH-forward-5′-AAG GGC ATC TTG GCC TAC AC, reverse-5′-CGG CGT ACA TGT GCT TGA TG

C/EBP-forward-5′-GCA ACT CAG CAA CGC AAC AAA TGC, reverse-5′-TTT AGC GGA GAT GTG GCA TGG AGT

CREB1α-forward-5′-GGA AAT CTT CAG ACG ATC CAA GTT, reverse-5′-TGT TTG GAC ATA TGA ATC GTG GC

CREB1 β-forward-5′-GAA GGC CTT CGT ACA GAT GTC C, reverse-5′-CGA CTG GTA TGT AAA ACT GTC CAT

Discussion

The results are preliminary since more data needs to be collected via qPCR. Therefore, the graphs containing C/EBP expression in the buccal and cerebral ganglia are not shown.

- Animals that were obvious outliers were not included in the graphs.
- Animals that showed more interest during training followed a general pattern of higher expression. The opposite was true for those who were less responsive to the food offered.

The species used, *Aplysia fasciata*, are nocturnal and learn less effectively during the day. Therefore, it is of interest to note that there was an increase in CREB1α expression although the Aplysia received training and lip stimulation during the day.

Future Work

- Running additional cDNA samples through a qPCR analysis will allow for a clearer vision of changes in the gene expression of trained animals.
- Since animals that were more interested in training had higher gene expression, a study correlating the interest in the training and the resulting gene expression would show a connection between the gene studied and memory formation.

Acknowledgements

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