MicroRNA Regulation due to Long-Term Memory

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Introduction

Memory = Retention of learned information

Understanding the molecular basis of memory can lead to:
- **Targeted therapies for memory disorders such as PTSD or Alzheimer's**
- **Prevention of memory disorders**

Key regulators of memory include:
- **cAMP Response Element Binding Protein 1 (CREB-1)[4]**
- **cAMP Response Element Binding Protein 2 (CREB-2)[4]**
- **Cytoplasmic polyadenylation element binding protein (CPEB)[7]**

PROTEIN/ TARGET SEQUENCE	ROLE IN LONG TERM MEMORY	REGULATION AFTER LONG TERM MEMORY
CREB-1	activates genes necessary for LTM[4]	Upregulation[4]
CREB-2	suppresses genes for LTM during basal conditions[4]	Downregulation[4]
CPEB	activates target RNA for local protein synthesis in synapse[9]	Upregulation[9]
microRNA-124	inhibitor of CREB-1 in basal conditions[8]	Downregulation[8]
microRNA-22	inhibitor of CPEB in basal conditions[9]	Downregulation[9,3]
Dicer	cleaves precursor RNA into mature RNA[8]	Downregulation (Hypothesized)

Recently, microRNAs have been implicated in the regulation of these key memory proteins.[2,3]

KEY QUESTION: How are microRNAs (miRs) regulated after long-term memory?

HYPOTHESIS: After the initiation of long-term memory, microRNAs levels are decreased through downregulation of Dicer, a protein which cleaves precursor miRs into mature miRs.[8]

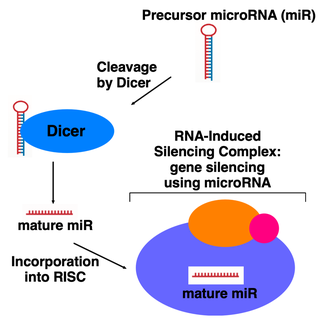


Figure 1. Steps in microRNA processing[6]

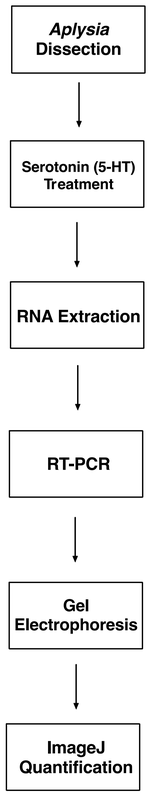
Aplysia Californica

- **Simple nervous system (20,000 neurons)**
- **Large neurons**
- **Identifiable neural circuits**
- **Simple genetics[6]**

Figure 2. *Aplysia californica*, a marine snail, offers a reductionist model system.[6]

Methods and Materials

Procedure



Serotonin treatment —> Long-term memory in isolated ganglia[1]

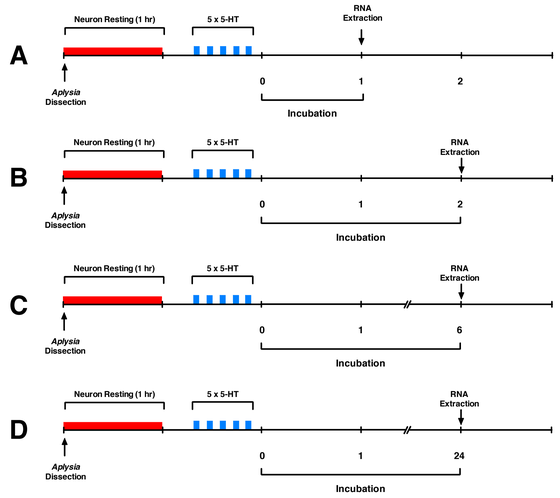


Figure 3. Separation of experimental groups by time of RNA extraction

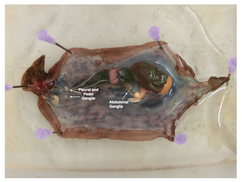


Figure 4. A dissected *Aplysia* with ganglia identified

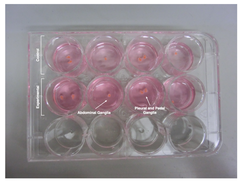


Figure 5. Serotonin is administered to isolated ganglia post-dissection.

Results

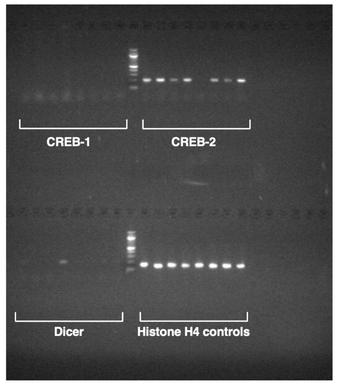
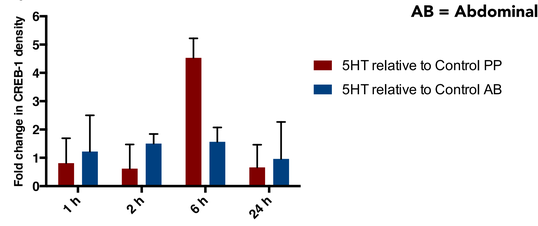


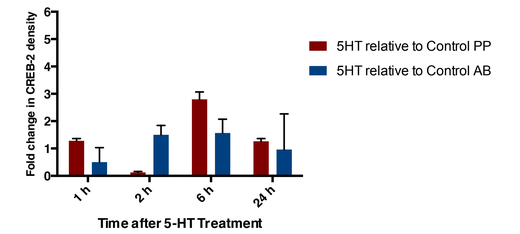
Figure 6. Gel electrophoresis is run in order to visualize PCR reactions. Gel image left shows samples with RNA extracted at time t=1. Histone H4 is used as a control.[10]

Comparison of CREB-1 levels in 5-HT vs. Control

PP = Pleural and Pedal Ganglia
AB = Abdominal Ganglia



Comparison of CREB-2 levels in 5-HT vs. Control



Comparison of Dicer levels in 5-HT vs. Control

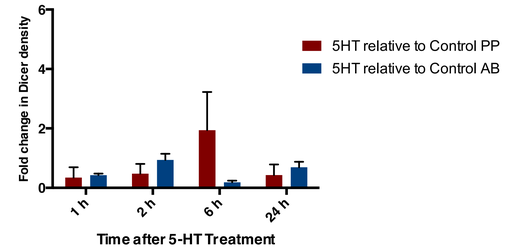


Figure 7. Levels of fluorescence in each sample were quantified and normalized to levels of Histone H4 for the same sample. Average density of fluorescence of each probe per sample is an indicator of mRNA levels. Density for each sample in the experimental groups was compared to the average density for the control group (with the same time, t)

Conclusions

The data from this study suggest that modulation of CREB-1, CREB-2 and Dicer is dynamic. However, this preliminary study, with a small sample size (*n*=2), is inconclusive.

Future Work

Examination of Dicer regulation at the protein level
- **→Western blot using an antibody probe for Dicer**
- **→Probes for active and inactive forms of Dicer**
- **→qPCR of mRNA**

Examination of Argonaute protein (Dicer-independent pathway for microRNA cleavage)[5]
- **→Quantification of mRNA levels of Argonaute protein after LTM**
- **→Western blot using antibody probe for Argonaute**

References

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