The role of microRNAs in memory consolidation in *Lymnaea*

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INTRODUCTION

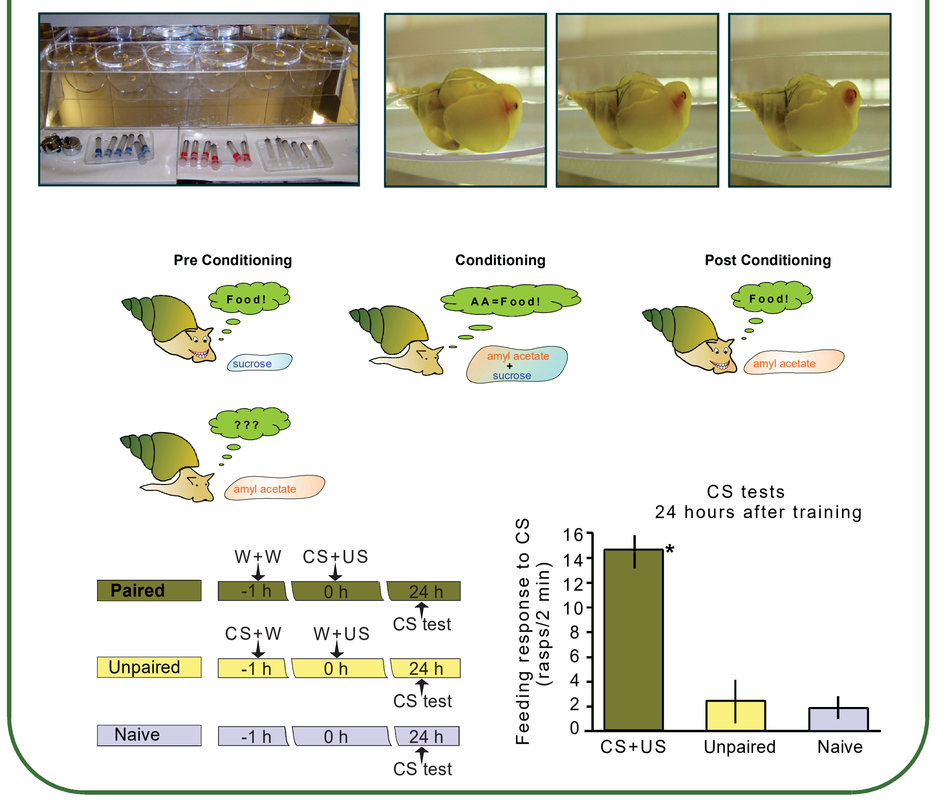
After single-trial classical conditioning (Fig. 1), there are well-defined time windows of activation of and requirement for key 'conventional' molecular players in the different phases of the consolidation of long-term memory (LTM) in *Lymnaea* (Figs. 2 and 3). Two important related discoveries we have made recently are:

i) late LTM (24h post-training) requires transcription at 6h post-training (Fig. 4A)

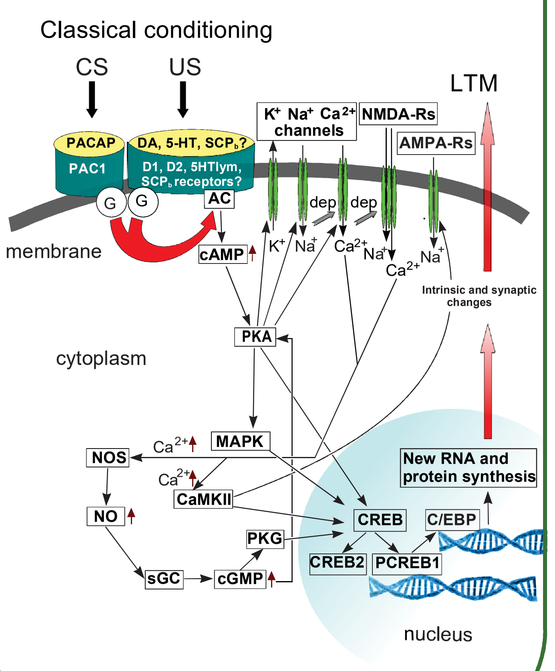
ii) at 6h post-training, there is ongoing phosphorylation of CREB1 and increased acetylation of H3, both of which can be measured in the 'learning ganglia' as well as in single identified neurons known to be involved in learning (Figure 4B, C).

However, the requirement for new protein synthesis for LTM only lasts for up to 1h after conditioning (Fig. 3). Together, these findings gave rise to the hypothesis that newly transcribed non-coding RNAs (e.g., miRNAs) are involved in the early as well as intermediate-term phase of memory consolidation. We tested this hypothesis by investigating the temporal dynamics of the post-training expression of miRNAs in the 'learning ganglia' of *Lymnaea*.

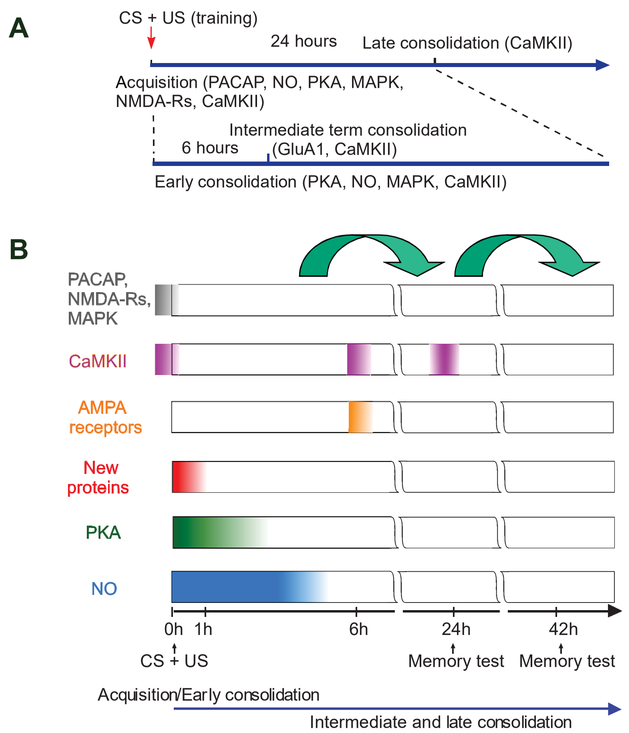
1 SINGLE TRIAL REWARD CLASSICAL CONDITIONING LEADING TO LTM IN *LYMNAEA*



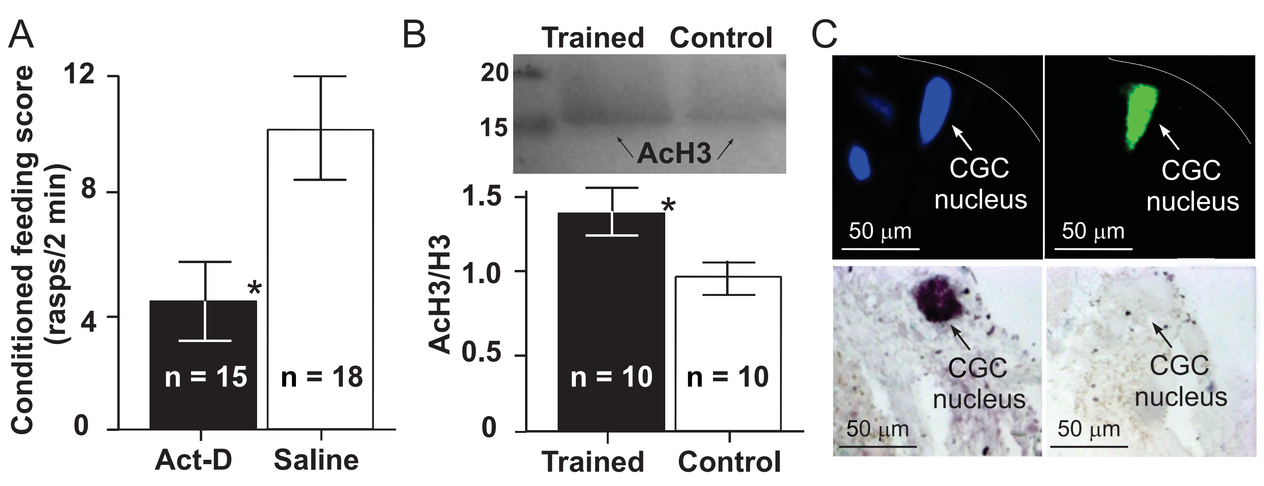
2 CONSERVED MOLECULAR PROCESSES OF MEMORY CONSOLIDATION IN *LYMNAEA*



3 TIME WINDOWS OF ACTIVATION OF (A) AND REQUIREMENT FOR KEY 'CONVENTIONAL' MOLECULAR PLAYERS (B) IN THE DIFFERENT PHASES OF THE CONSOLIDATION OF LTM IN *LYMNAEA*



4 ONGOING GENE TRANSCRIPTION AT 6H AFTER SINGLE-TRIAL TRAINING IN *LYMNAEA*



(A) For LTM at 24h, transcription is required at 6h post-training.

(B) Increased AcK18H3 levels in nuclear extracts from the 'learning ganglia' at 6h post-training, measured in western blots. The loading control was total H3.

(C) AcK18H3 signal (green) in the CGC nucleus at 6h post-training. Other DAPI-stained neuronal nuclei (blue) in the same CGC's vicinity do not show the AcH3 signal. pS133 CREB is also upregulated in the CGC nucleus at 6h after training (bottom left panel) compared to control (bottom right panel).

5 CONSERVED DIFFERENTIALLY EXPRESSED (X) miRNAs IN THE *LYMNAEA* 'LEARNING GANGLIA' (A) AND THEIR POTENTIAL RNA TARGETS (B, C)

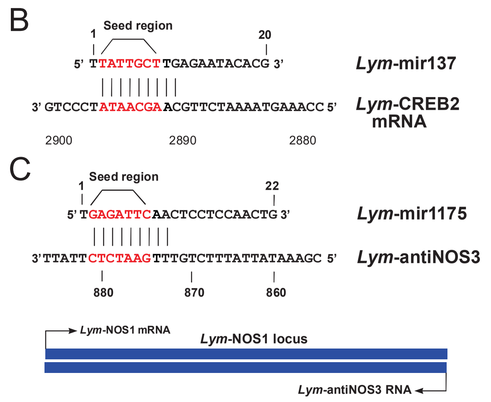
sncRNA cDNA libraries were produced from the 'learning ganglia' dissected from experimental animals at 1h, 6h or 24h after a single conditioning trial, corresponding to the early, intermediate and late phases of memory consolidation. Next Generation Sequencing of the libraries and bioinformatic analysis revealed over a hundred individual sncRNAs with significant homology to miRNAs previously identified in other species, including C. elegans, *Drosophila, Aplysia*, mouse and humans.

A

	Type of microRNA	Time after training 1 h	6 h	24 h
1	miR-1	x	x	
2	miR-2	x	x	x
3	miR-7	x	x	x
4	miR-8	x	x	x
5	miR-29	x		
6	miR-31	x		x
7	miR-67	x	x	x
8	miR-71	x	x	x
9	miR-72	x	x	
10	miR-87	x		x
11	miR-96	x		x
12	miR-98	x		x
13	miR-99	x	x	x
14	miR-100	x	x	x
15	miR-137	x		x
16	miR-153		x	
17	miR-184	x	x	x
18	miR-190	x	x	x
19	miR-210	x	x	x
20	miR-216	x	x	x
21	miR-277	x		
22	miR-278	x	x	x
23	miR-279		x	x
24	miR-317	x		
25	miR-375		x	x
26	miR-745	x	x	x
27	miR-750	x	x	x
28	miR-981	x		
29	miR-1175	x		
30	miR-1984	x	x	x
31	miR-1985	x	x	x
32	miR-1994		x	
33	miR-2001	x		x
34	miR-2722	x	x	
35	miR-3596	x	x	x

Grey: 'common' miRNAs
Blue and pink: 'phase-specific' miRNAs
White: miRNAs detected in two different phases of memory consolidiation.

B



(B) Target region for *Lym*-mir137 in the mRNA of CREB2, repressor of the transcriptional activator CREB1. (C) A long non-coding natural antisense transcript (NAT) is a potential target for *Lym*-mir1175. The seed region of *Lym*-mir1175 exhibits perfect sequence complementarity to a target site within the 3' UTR of *Lym*-antiNOS3 RNA. *Lym*-antiNOS3 RNA is a long non-coding NAT produced from the non-template strand of the *Lym*-NOS1 locus. *Lym*-antiNOS3 RNA is likely to serve as a negative regulator of NOS1 expression.

CONCLUSIONS

- We identified individual annotated miRNAs belonging to 35 conserved miRNA families exhibiting learning-induced changes in their expression. Over 50% of these miRNAs showed differential changes in their expression at different times after training. Five miRNA families are specific for the 1h post-training group and 2 are specific for the 6h post-training group. The rest of the miRNAs were differentially expressed in two phases of consolidation and no miRNAs specific for the 24h post-training group have been found.
- Many of the differentially expressed miRNAs are known to be involved in neuronal functions, including 14 that are also present in the human brain and 24 that are present in the *Aplysia* CNS.
- We have also identified potential targets for two of the differentially expressed miRNAs (*Lym*-miR137 and *Lym*-miR1175) identified in these experiments, *Lym*-CREB2-encoding mRNA and the long non-coding RNA *Lym*-antiNOS3, respectively. Notably, both CREB2 and NOS are important components of conventional mechanisms involved in memory formation. We propose that *Lym*-miR137 inhibits the translation of CREB2 whereas *Lym*-miR1175 promotes the translation of NOS.

References

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