Figure S1

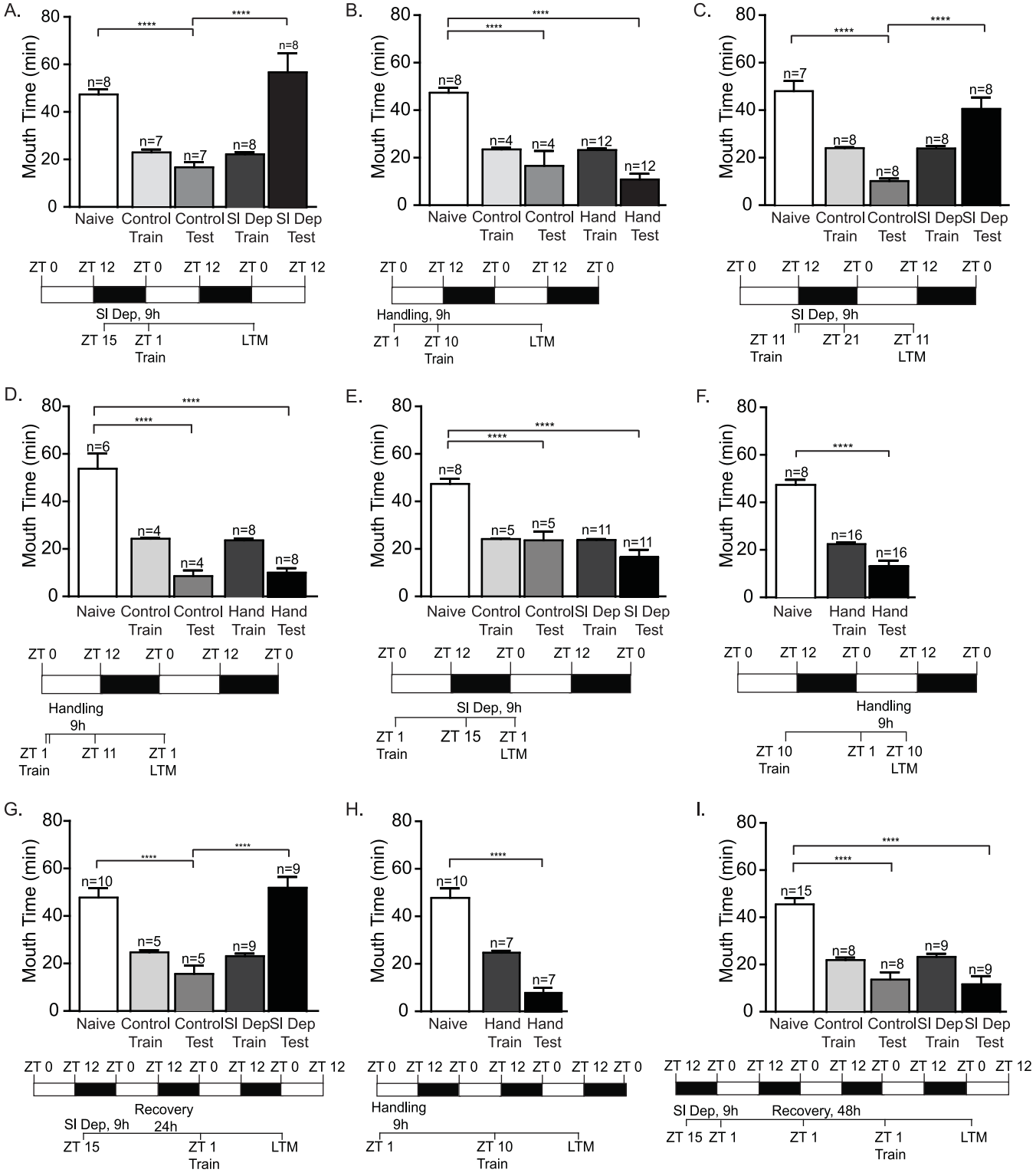
A.
Mouth Time (min)
Naive
Control Train
Control Test
SI Dep Train
SI Dep Test
SI Dep, 9h
ZT 15
ZT 1 Train
LTM
B.
Naive
Control Train
Control Test
Hand Train
Hand Test
Handling, 9h
ZT 1
ZT 10 Train
LTM
C.
Naive
Control Train
Control Test
SI Dep Train
SI Dep Test
SI Dep, 9h
ZT 11 Train
ZT 21
ZT 11 LTM
D.
Naive
Control Train
Control Test
Hand Train
Hand Test
Handling 9h
ZT 1 Train
ZT 11
ZT 1 LTM
E.
Naive
Control Train
Control Test
SI Dep Train
SI Dep Test
SI Dep, 9h
ZT 1 Train
ZT 15
ZT 1 LTM
F.
Naive
Hand Train
Hand Test
Handling 9h
ZT 10 Train
ZT 1
ZT 10 LTM
G.
Naive
Control Train
Control Test
SI Dep Train
SI Dep Test
Recovery 24h
SI Dep, 9h
ZT 15
ZT 1 Train
LTM
H.
Naive
Hand Train
Hand Test
Handling 9h
ZT 1
ZT 10 Train
LTM
I.
Naive
Control Train
Control Test
SI Dep Train
SI Dep Test
SI Dep, 9h
Recovery, 48h
ZT 15
ZT 1
ZT 1 Train
LTM

Figure S1—The effects of sleep deprivation on long-term memory with analysis of a second parameter of memory: total time of seaweed retention in the mouth. A) LTM induction was blocked after 9 h acute sleep deprivation. Data presented in this panel corresponds to Figure 1A. Sleep deprived trained animals (Sl Dep Test) retained the seaweed in the mouth with times similar to naïve animals and significantly greater times with respect to non-sleep deprived trained animals (Control Test; one-way ANOVA $F_{(4,33)} = 19.14$, $P < 0.0001$). Data analysis was performed using ANOVA followed by Bonferroni's MCT. Asterisks denote significant differences with ****$P < 0.0001$. B) During testing for LTM induction after 9 h of gentle handling, trained day-time handled control animals (Hand Test) exhibited significant decreases in the time the seaweed was retained in the mouth compared to naïve animals indicating robust long-term memory, similar to the decreases seen in trained non-handled animals (Control Test; one-way ANOVA $F_{(4,35)} = 37.96$, $P < 0.0001$). Asterisks represent post- hoc analyses ****$P < 0.0001$ for testing between naïve and non-handled control animals and between naïve animals and handled animals. Data presented in this panel corresponds to Figure 1B. C) Acute sleep deprivation for 9 h blocked consolidation of LTM. Data presented in this panel corresponds to Figure 2A. Sleep deprived animals exhibited mouth times similar to Naïves and significantly different from non-sleep deprived animals (one-way ANOVA $F_{(4,34)} = 26.33$, $P < 0.0001$). Asterisks represents Bonferroni's post-hoc analyses ****$P < 0.0001$ for testing between naïve and non-sleep deprived animals and between non-sleep deprived and sleep deprived animals. D) During testing for LTM consolidation, trained day-time, handled control animals exhibited significantly shorter times the seaweed was retained in the mouth similar to non-handled trained animals and were significantly different than naïves (one-way ANOVA $F_{(4,25)} = 31.11$, $P < 0.0001$). Asterisks represent Bonferroni's post-hoc analyses ****$P < 0.0001$

for testing between naïve and non-handled control animals and between naïves and handled animals. Data in this panel corresponds with Figure 2B. E) Sleep deprivation did not block recall of memory. Trained sleep deprived animals showed a significant decrease in mouth time when compared to naive animals (one-way ANOVA $F_{(4,35)} = 24.35$ $P < 0.0001$). Asterisks represents Bonferroni's post-hoc analyses ****P <0.0001 for testing between naïve and trained non-sleep deprived animals and between naïves and trained sleep deprived animals. Data in this panel corresponds to Figure 3A. F) During testing for recall of memory, trained day-time handled control animals (Hand Test) had significantly decreased mouth times compared to naïve animals (one-way ANOVA $F_{(2,37)} = 67.63$, $P < 0.0001$). Asterisks represent Bonferroni's post-hoc analyses ****P <0.0001 for testing between naïve and handled control animals. Data in this panel corresponds to Figure 3B. G) Sleep deprivation persistently inhibited LTM induction for at least 24 h. Data in this panel corresponds to Figure 4A. Trained sleep-deprived animals (Sl Dep Test) were significantly different from the trained non-sleep deprived animals (Control Test) and exhibited mouth times similar to naïve animals. (one-way ANOVA $F_{(4,33)} = 19.78$, $P < 0.0001$). Asterisks represents Bonferroni's post-hoc analyses ****P <0.0001 for testing between naïve and non-sleep deprived animals and between non-sleep deprived and sleep deprived animals. H) Trained day-time handled control animals (Hand Test) had significantly decreased retention of the seaweed in the mouth compared to naïve animals indicating long-term memory. (one-way ANOVA $F_{(2,21)} = 43.58$, $P < 0.0001$). Asterisks represents Bonferroni's post-hoc analyses ****P <0.0001 for testing between naïve and trained handled animals. Data in this panel corresponds to Figure 4B. I) The persistent effect of sleep deprivation on LTM induction was ameliorated after 48 h of recovery. Trained sleep deprived animals showed a significant decrease in mouth times when compared to naive animals (one-way ANOVA $F_{(4,44)} = 31.89$, P

< 0.0001). Asterisks represents Bonferroni's post-hoc analyses ****P <0.0001 for testing between naïve and non-sleep deprived animals and between naïves and sleep deprived animals. Data in this panel corresponds to Figure 4C.

Figure S2

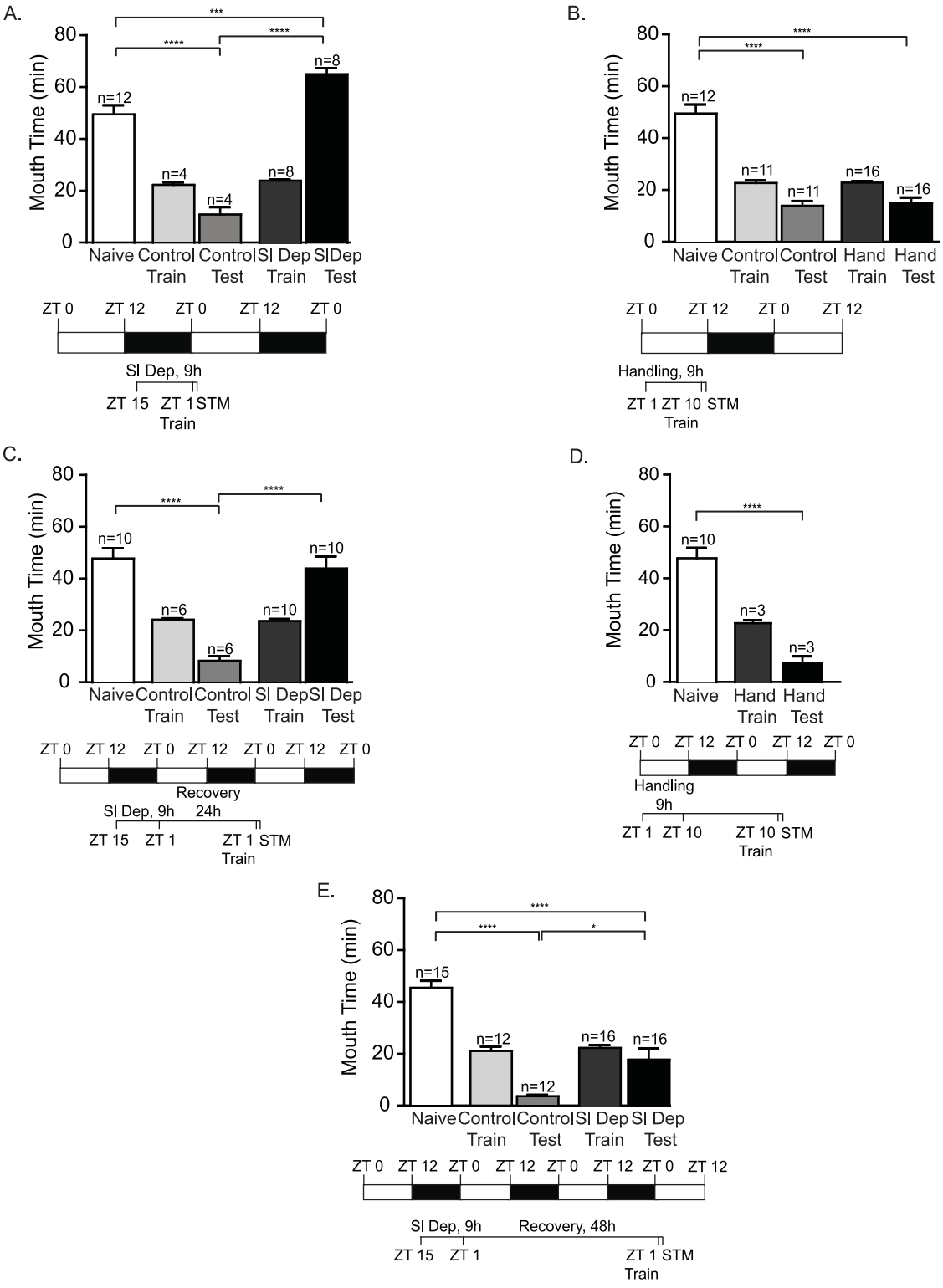
A.
Mouth Time (min)
n=12
n=4
n=4
n=8
n=8
Naive
Control Train
Control Test
SI Dep Train
SI Dep Test
ZT 0 ZT 12 ZT 0 ZT 12 ZT 0
SI Dep, 9h
ZT 15 ZT 1
STM Train
B.
Mouth Time (min)
n=12
n=11
n=11
n=16
n=16
Naive
Control Train
Control Test
Hand Train
Hand Test
ZT 0 ZT 12 ZT 0 ZT 12
Handling, 9h
ZT 1 ZT 10
STM Train
C.
Mouth Time (min)
n=10
n=6
n=6
n=10
n=10
Naive
Control Train
Control Test
SI Dep Train
SI Dep Test
ZT 0 ZT 12 ZT 0 ZT 12 ZT 0 ZT 12 ZT 0
Recovery
SI Dep, 9h
24h
ZT 15 ZT 1
ZT 1
STM Train
D.
Mouth Time (min)
n=10
n=3
n=3
Naive
Hand Train
Hand Test
ZT 0 ZT 12 ZT 0 ZT 12 ZT 0
Handling
9h
ZT 1 ZT 10
ZT 10
STM Train
E.
Mouth Time (min)
n=15
n=12
n=12
n=16
n=16
Naive
Control Train
Control Test
SI Dep Train
SI Dep Test
ZT 0 ZT 12 ZT 0 ZT 12 ZT 0 ZT 12 ZT 0 ZT 12
SI Dep, 9h
Recovery, 48h
ZT 15 ZT 1
ZT 1
STM Train

Figure S2—Mouth times for short-term memory: A) STM induction was blocked after 9 h acute sleep deprivation. Data in this panel corresponds to Figure 5A. Trained sleep-deprived animals (Sl Dep Test) exhibited significantly longer retention of the seaweed in the mouth compared to the trained non-sleep deprived animals (Control Test) and exhibited mouth times similar to naïve animals (one-way ANOVA $F_{(4,31)} = 48.74$, $P < 0.0001$) Asterisks represents Bonferroni's post-hoc analyses ****P <0.0001 for testing between naïves and non- sleep deprived animals and between non-sleep deprived and sleep deprived animals. ***P<0.001 for testing between naïves and sleep deprived animals. B) During testing for STM induction after 9 h of day-time handling, trained handled animals (Hand Test) exhibited short mouth times similar to trained non-handled animals (Control Test) that were significantly different than naïve animals (one-way ANOVA $F_{(4,61)} = 39.39$, $P < 0.0001$) Asterisks represents Bonferroni's post-hoc analyses ****P <0.0001. Data in this panel corresponds to Figure 5B. C) Sleep deprivation persistently inhibited STM for 24 h. Data in this panel corresponds to Figure 6A. Trained sleep-deprived animals (Sl Dep Test) had significantly longer times the seaweed was retained in the mouth compared to trained non-sleep deprived animals (Control Test) with mouth times similar to naïve animals (one-way ANOVA $F_{(4,37)} = 21.75$, $P < 0.0001$) Asterisks represents Bonferroni's post-hoc analyses ****P <0.0001. D) Daytime handling did not affect memory formation. Data in this panel corresponds to Figure 6B. Trained day-time handled control animals (Hand Test) had significantly shorter mouth times compared to naïve animals (one-way ANOVA $F_{(2,13)} = 19.34$, $P < 0.0001$) indicating memory. Asterisks represent Bonferroni's post-

hoc analyses ****P < 0.0001. E) The persistent effects of sleep deprivation on STM induction was mitigated after 48 h of recovery. Data in this panel corresponds to Figure 6C. Trained sleep deprived animals showed a significant decrease in mouth times compared to naive animals (one- way ANOVA $F_{(4,66)} = 30.22$, $P < 0.0001$). Asterisks represent Bonferroni's post-hoc analyses

****P <0.001 for testing between naive and non-sleep deprived animals and between naive and sleep deprived animals. *P<0.05 for testing between non-sleep deprived and sleep deprived animals.

Figure S3

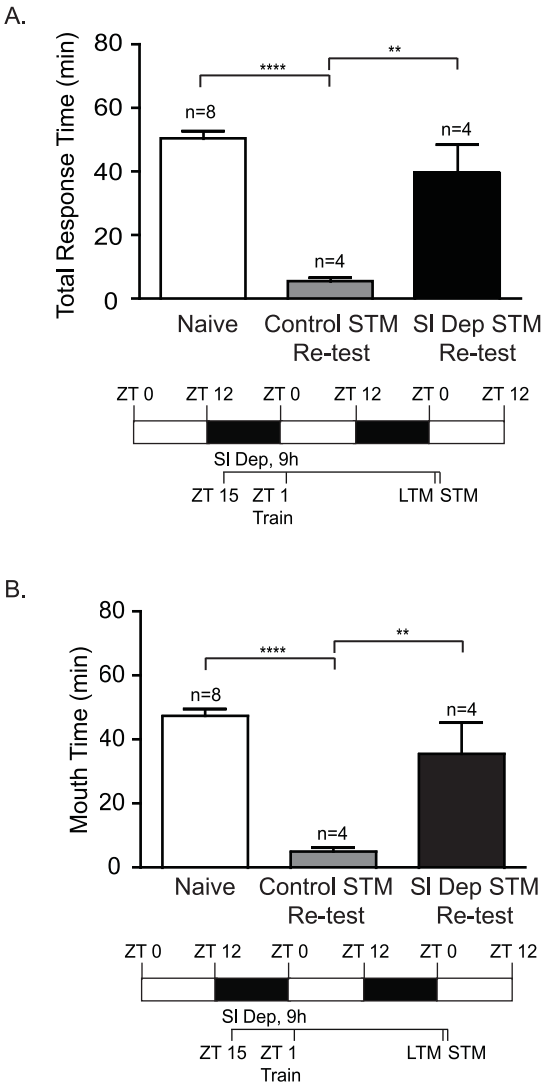
A.
Total Response Time (min)
80
60
40
20
0
****
**
n=8
n=4
n=4
Naive
Control STM Re-test
SI Dep STM Re-test
ZT 0
ZT 12
ZT 0
ZT 12
ZT 0
ZT 12
SI Dep, 9h
ZT 15
ZT 1
Train
LTM STM
B.
Mouth Time (min)
80
60
40
20
0
****
**
n=8
n=4
n=4
Naive
Control STM Re-test
SI Dep STM Re-test
ZT 0
ZT 12
ZT 0
ZT 12
ZT 0
ZT 12
SI Dep, 9h
ZT 15
ZT 1
Train
LTM STM

Figure S3—Over-training does not rescue STM immediately after sleep deprivation.

(A) To determine whether additional training mitigated the effects of sleep deprivation, animals were re-tested for STM 30 min after the initial LTM test shown in Figure 1A. As the testing session is procedurally identical to training, the LTM test acted as a second training session. The animals that failed to exhibit LTM were retested for STM 30 min later. Two training sessions did not rescue STM (one-way ANOVA $F_{(2,13)} = 26.88$, $P < 0.0001$).

Asterisks represents Bonferroni's post-hoc analyses ****P <0.0001 for testing between naïve and non-sleep deprived animals and **P<0.01 for testing between non-sleep deprived and sleep deprived animals. (B) Trained sleep-deprived (Sl Dep STM Re-test) animals exhibited significantly longer retention of the seaweed in the mouth compared to the non-sleep deprived trained animals (Control STM Re-test) and exhibited mouth times similar to naïve animals (one- way ANOVA $F_{(2,13)} = 22.44$, $P < 0.0001$). Asterisks represents Bonferroni's post-hoc analyses

****P <0.0001 for testing between naïve and non-sleep deprived animals and **P<0.01 for testing between non-sleep deprived and sleep deprived animals.