Increase in cyclic AMP concentration in a cerebral giant interneuron mimics part of a memory trace for conditioned taste aversion of the pond snail

Emi Otsuka¹, Miho Matsunaga¹, Ryuichi Okada¹, Miki Yamagishi¹, Akiko Okuta², Ken Lukowiak³ and Etsuro Ito¹

¹Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Sanuki, Kagawa 769-2193, Japan
²Cellular and Structural Physiology Institute, Nagoya University, Nagoya, Aichi 464-8601, Japan
³Hotchkiss Brain Institute, University of Calgary, Calgary, AB T2N 4N1, Canada

Received June 20, 2013; accepted October 18, 2013

Conditioned taste aversion (CTA) can be classically conditioned in the pond snail Lymnaea stagnalis and subsequently be consolidated into long-term memory (LTM). The neural trace that subserves CTA-LTM can be summarized as follows: A polysynaptic inhibitory postsynaptic potential recorded in the neuron 1 medial (N1M) cell in the conditioned snails as a result of activation of the cerebral giant cell (CGC) is larger and lasts longer than that in control snails. The N1M cell is ultimately activated by the CGC via the neuron 3 tonic (N3t) cell. That is, the inhibitory monosynaptic inputs from the N3t cell to the N1M cell are facilitated. The N1M and N3t cells are the members of feeding central pattern generator, whereas the CGC is a multimodal interneuron thought to play a key role in feeding behavior. Here we examined the involvement of a second messenger, cAMP, in the establishment of the memory trace. We injected cAMP into the CGC and monitored the potentials of the B3 motor neuron activated by the CGC. B3 activity is used as an index for the synaptic inputs from the N3t cell to the N1M cell. We found that the B3 potentials were transiently enlarged. Thus, when the cAMP concentration is increased in the CGC by taste aversion training, cAMP-induced changes may play a key role in the establishment of a memory trace in the N3t cell.

Key words: cAMP, conditioned taste aversion, feeding, Lymnaea, memory trace

The pond snail Lymnaea stagnalis has the ability to learn and remember to avoid specific tastes. This phenomenon is known as conditioned taste aversion (CTA)¹⁻³. To produce CTA, an appetitive stimulus (e.g., sucrose) is used as the conditioned stimulus (CS). Application of the CS to the lips increases the feeding response (i.e., the number of bites) in snails. An aversive stimulus (e.g., KCl) is used as the unconditioned stimulus (US). Application of the US to the snails induces a withdrawal response into the shell, resulting in inhibiting feeding behavior. In the taste aversion-training procedure, the CS is paired with the US. After repeated temporal contingent presentations of the CS and US, the CS no longer elicits a feeding response, and this taste aversion persists for more than a month¹.

The neural mechanisms underlying CTA in Lymnaea have been examined⁴⁻⁷. The cerebral giant cells (CGCs) act as a pair of multimodal interneurons that play key roles in the mediation of learning and memory of feeding behaviors⁴⁻¹². We showed that both the CS and US used in taste aversion-training alter the activity of the CGCs⁴⁻¹². Based on the results from Paul Benjamin’s laboratory⁴, we focused our experiments on a polysynaptic inhibitory postsynaptic potential (IPSP) recorded in the neuron 1 medial (N1M) cell by activation of the CGC via the neuron 3 tonic (N3t) cell. This IPSP was larger and lasted longer in the taste aversion-trained snails than that in the control snails¹⁶. These data are...
consistent with the hypothesis that an enhanced IPSP to the N1M cell underlies the suppression of feeding response in CTA of Lymnaea.

To determine whether the enhancement of this IPSP to the N1M cell in the taste-aversion trained snails is the result of a change in properties of the CGC, the electrical properties of the CGC were compared between the taste aversion-trained snails and the control snails. No significant differences were found in the resting membrane potential, the input resistance, the half width of spontaneous action potential, the half width of after-hyperpolarization of spontaneous action potential, and the threshold for an action potential. We therefore examined the changes in the postsynaptic N1M cell. No significant differences were either found in the resting potential between the taste aversion-trained snails and the control snails. These results suggested to us that CTA of Lymnaea is the result of a memory trace in the N3t cell. This neuron receives an excitatory monosynaptic input (i.e., EPSP) from the CGC and connects to the N1M cell by means of a monosynaptic IPSP.

We have monitored N3t activity following the establishment of CTA. However, the N3t cell is too small to be able to consistently record its activity via standard sharp electrode recording techniques. Thus, the synaptic inputs from the CGC to the N3t cell and those from the N3t cell to the N1M cell are inferred by monitoring the EPSPs recorded in the large B1 and B3 motor neurons, respectively (Fig. 1). The spontaneous EPSPs recorded in the B3 motor neuron were significantly enlarged in taste-aversion trained snails compared to control snails. These data suggested that, after taste aversion training, the monosynaptic inputs from the N3t cell to its follower neurons, including the N1M cell, are facilitated. That is, a neural correlate of CTA in Lymnaea is an increase in neurotransmitter release from the N3t cell. Thus, in taste aversion-trained snails the CS causes the N3t cell to suppresses activity in the N1M cell and suppresses the feeding central pattern generator (CPG).

In general, the molecular mechanisms underlying long-term memory (LTM) consolidation are thought to be mediated by cAMP signaling cascades across phyla in the animal kingdom. It is thought that cAMP activation mediates LTM mainly by activating the cAMP-sensitive protein kinase A (PKA) that can then phosphorylate various downstream kinases and transcription factors required for LTM. Although we have no direct data showing that the cAMP concentration is increased in the CGC by the taste-aversion training procedure, our previous results have led us to hypothesize that if cAMP is increased in the CGC, such cAMP cascades might result in a change in activity that resembles the changes seen following CTA training. That is, cAMP cascades may result in predictable changes not only in the CGC-N3t pathway (i.e., the B1 activity) but also in the N3t-N1M pathway (i.e., the B3 activity). In the present study, we show that causing an increase in cAMP concentration in the CGC activates the N3t-N1M pathway as evidenced by alterations in the synaptic input to the B3 motor neuron.

Materials and Methods

CNS preparations

The pond snails Lymnaea stagnalis with a 15–25 mm shell (young adults), originally supplied from Vrije Universiteit Amsterdam, were maintained in dechlorinated tap water (i.e., pond water) under a 12:12 light-dark cycle at 20°C and fed ad libitum on a kind of turnip leaf Brassica rapa var. peruviridis (Komatsuna [in Japanese]) and a spiral shell food (Nisso, Saitama, Japan) every other day. Snails were anesthetized with 25% Listerine® before dissection. The isolated central nervous system (CNS) was immersed in Lymnaea saline and pinned in a Sylgard®-lined dish for electrophysiological recording. Lymnaea saline contained: 50 mM NaCl, 1.6 mM KCl, 2.0 mM MgCl₂, 3.5 mM CaCl₂, 10 mM HEPES (pH 7.9).

Intracellular recording

The CGC, the B1 motor neuron and the B3 motor neuron were impaled with glass microelectrodes filled with 2 M potassium acetate giving tip resistances of 20–50 MΩ. A train of ca. 10 spikes in the CGC, which was produced by a current injection (1.1–1.5 nA) for 1 s, evoked a single large compound EPSP both in the B1 motor neuron and the B3 motor neuron (electric stimulator: SEN-7203, Nihon Kohden, Tokyo, Japan; intracellular recording amplifier: MEZ-8300, Nihon Kohden; AD converter: Digidata 1322A, Axon Instruments, Foster City, CA, USA). We used the neurons...
located in the ipsilateral side. Cyclic AMP (Sigma-Aldrich, St. Louis, MO, USA), which was filled in a glass micro-electrode (80–120 MΩ) as a 200 mM solution dissolved in 20 mM Tris buffer (pH 7.5), was injected into the CGC by passing hyperpolarizing current pulses (50 ms on, 50 ms off) of 4 nA for 20 min. The injection period was decided by conferring with the work of Aplysia\textsuperscript{26}. Before and after the injection of hyperpolarizing current into the CGC, the EPSP recorded in the B1 and B3 motor neurons is not changed by activation of the CGC\textsuperscript{22,23}. The data were recorded in the same preparation at the following 4 time points after cAMP injection: 1, 3 and 6 h. The data at 0 h were recorded before cAMP injection. For estimation of EPSP changes recorded in the B1 and B3 motor neurons, the area of EPSP was calculated. That is, the unit for Figures 2B and 3B is arbitrary.

Statistics
The data are expressed as the mean±SEM. Significant differences at $P<0.05$ between 2 groups were examined by Student paired t-test. Significant differences at $P<0.05$ among 3 groups were examined by two-way repeated measure ANOVA and post hoc Scheffé test.

Results and Discussion
Recent studies on appetitive conditioning of feeding behavior in Lymnaea have elucidated three points\textsuperscript{19}. (A) Tonic inhibition in the feeding network is provided by the N3t cell. This interneuron makes a monosynaptic inhibitory connection (IPSP) to the N1M cell. (B) There is a reduction in N3t spiking after appetitive conditioning, and this reduction in N3t firing inversely correlates with an increase in the conditioned fictive feeding response. (C) Computer simulation of N3t-N1M interactions suggests that changes in N3t firing are sufficient to explain the increase in the fictive feeding activity produced by appetitive conditioning. These data showed that appetitive conditioning of feeding behavior in Lymnaea occurs due to the combined effects of reduced tonic inhibition and enhanced excitatory synaptic connections between the CS pathway and feeding command neurons.

These afore mentioned findings led us to hypothesized that ‘taste aversion learning’ would occur via a mechanism that was the inverse of the mechanism proposed for ‘appetitive conditioning’. That is, there would be an increase in N3t spiking after conditioning, and this increase in N3t firing would inversely correlate with a reduction in the conditioned fictive feeding response. However, as described in the first section, because the N3t cell is too small to consistently record from using standard sharp electrode recording techniques, we inferred the synaptic inputs from the CGC to the N3t cell and those from the N3t cell to the N1M cell by monitoring the EPSPs recorded in the large B1 and B3 motor neurons, respectively (Fig. 1)\textsuperscript{18}.

Both the CS and the US elicit activity in the CGC during taste aversion training\textsuperscript{14,15}. However, we have no data showing that the cAMP concentration is increased in the CGC as a result of taste aversion training, because no method is applicable to single-cell cAMP measurement. Previous studies, however, showed that if the cAMP concentration was increased in the CGC by injection of cAMP, the EPSP recorded in the B1 motor neuron as a result of depolarization of the CGC was enlarged\textsuperscript{22,23}. Here, we replicated those previous findings (Fig. 2A). That is, the EPSP recorded in the B1 motor neuron was enlarged after injection of cAMP into the CGC (Fig. 2B). This enlargement of EPSP in the B1 motor neuron is associated with the increase in the amount of serotonin release from the CGC\textsuperscript{22,27}. We consider that this enlargement of EPSP in the B1 motor neuron is equivalent to the facilitation of the synaptic inputs from the CGC to the N3t cell.

Our most important finding in the present study is that
when the cAMP concentration was increased in the CGC, the EPSP recorded in the B3 motor neuron after depolarization of the CGC was significantly larger (Fig. 3). This enlargement occurred transiently 3 h after injection of cAMP into the CGC, thus the synaptic inputs from the N3t cell to the N1M cell were facilitated transiently. To our knowledge, there is no monosynaptic connection between the CGC and the B3 motor neuron. Therefore, the injection of cAMP into the CGC whereas directly enhancing synaptic connections made by the CGC indirectly alters connections made by neurons downstream of the CGC (i.e., interneurons in the feeding CPG including the N3t cell and the synaptic connections between the N3t cell to the N1M cell).

Previous studies have shown that in the CNS isolated from the taste-aversion trained snails the EPSPs recorded in the B1 motor neuron as a result of activation of the CGC were identical to those in the control snails, whereas the spontaneous EPSPs recorded in the B3 motor neuron (i.e., no stimulation for the CGC) were significantly enlarged. Our present data showed that a cAMP injection into the CGC increased the B3 activity, i.e., the N3t activity. We therefore conclude that an increase in cAMP concentration in the CGC mimics part of the memory trace in the feeding neural network for CTA in Lymnaea. We still have an issue to be addressed. After taste aversion training, only the B3 motor neuron was activated (i.e., N3t cell was activated) but the B1 motor neuron was not. This issue should be considered in the near future because the function of second messengers is not so simple.

A further issue to be addressed is how the transient enhancement of synaptic activity in the N3t-N1M pathway can be stabilized into a persistent LTM. That is, an increase in cAMP in the CGC solely is not sufficient to explain the long-lasting enhancement of synaptic activity of the N3t-N1M pathway following successful CTA-LTM in Lymnaea. A possible key molecule that is necessary for the establishment of the long-lasting neuronal memory trace is ‘insulin’. Previous studies have shown that molluscan insulin-related peptides (MIPs) were up-regulated in snails exhibiting CTA. Recently, when we applied MIPs to the isolated CNS, we observed a long-term change in synaptic enhancement of the synaptic connection between the CGC and the B1 motor neuron. We further examined whether the observed changes in synaptic plasticity were the result of pre- and/or postsynaptic alterations using the paired pulse procedure. The paired pulse ratio was unaltered following insulin application, suggesting that insulin’s effects on synaptic plasticity are mediated postsynaptically in the B1 motor neuron. Thus, it was suggested that both the postsynaptic changes due to insulin’s actions and the presynaptic plasticity by cAMP cascades are needed for the neural correlate for LTM.

The molecular cascades following to cAMP in the CGC are thought to include PKA, cAMP-responsive element binding protein (CREB) and CCAAT/enhancement binding protein (C/EBP). In particular, we note that the mRNA copy number of CREB repressor (CREB2) is tens to hundreds in a single CGC, whereas that of CREB activator (CREB1) was below the detection limits of the assay. These results suggested that the CREB cascade is regulated by an excess amount of CREB2 in the CGC.

**Conclusion**

In the present study, we showed that when the cAMP concentration is increased in the CGC by taste aversion training for Lymnaea, cAMP-induced changes may play a key role in the establishment of a memory trace in the N3t cell. Although we focused our attention on only cAMP as a second messenger, we also have to keep in mind that alter-
ations in the level of calcium occur in the CGCs following taste aversion training. We have observed an increase in calcium in the CGC with pairing of the CS and US (Ito et al., unpublished data). A rise in intracellular calcium concentration is most likely also important in learning and memory for feeding behaviors in Lymnaea.

Acknowledgments

This work was supported by KAKENHI from JSPS (Nos. 24657055 and 25291074 to E.I.) and by a grant from CIHR (No. MOP 64339 to K.L.).

References

1. Kojima, S., Yamanaka, M., Fujito, Y. & Ito, E. Differential neuroethological effects of aversive and appetitive reinforcing stimuli on associative learning in Lymnaea stagnalis. Zool. Sci. 13, 803–812 (1996).

2. Kawai, R., Sunada, H., Horikoshi, T. & Sakakibara, M. Conditioned taste aversion training. We have observed an increase in cal-

3. Kita, S., Hashiba, R., Ueki, S., Kimoto, Y., Abe, Y., Gotoda, Y., Suzuki, R., Uraki, E., Nara, N., Kanazawa, A., Hatakeyama, D., Kawai, R., Fujito, Y., Lukowiak, K. & Ito, E. Does conditioned taste aversion learning in the pond snail Lymnaea stagnalis produce conditioned fear? Biol. Bull. 220, 71–81 (2011).

4. Ito, E., Kobayashi, S., Kojima, S., Sadamoto, H. & Hatakeyama, D. Associative learning in the pond snail, Lymnaea stagnalis. Zool. Sci. 16, 711–723 (1999).

5. Ito, E., Kojima, S., Lukowiak, K. & Sakakibara, M. From likes to dislikes: conditioned taste aversion in the pond snail Lymnaea stagnalis. Can. J. Zool. 91, 405–412 (2013).

6. Yamanaka, M., Sadamoto, H., Hatakeyama, D., Nakamura, H., Kojima, S., Kimura, T., Yamashita, M., Urano, A. & Ito, E. Developmental changes in conditioned taste aversion in Lymnaea stagnalis. Zool. Sci. 16, 9–16 (1999).

7. Yamanaka, M., Hatakeyama, D., Sadamoto, H., Kimura, T. & Ito, E. Development of key neurons for learning stimulates learning ability in Lymnaea stagnalis. Neurosci. Lett. 278, 113–116 (2000).

8. McCoohan, C. R. & Benjamin, P. R. Synaptic relationships of the cerebral giant cells with motor neurones in the feeding system of Lymnaea stagnalis. J. Exp. Biol. 85, 169–186 (1980).

9. Kyriakides, M. A. & McCoohan, C. R. Effect of putative neuromodulators on rhythmic buccal motor output in Lymnaea stagnalis. J. Neurobiol. 26, 635–650 (1999).

10. Elliott, C. J. & Vehovszky, A. Comparative pharmacology of feeding in molluscs. Acta Biol. Hung. 51, 153–163 (2000).

11. Straub, V. A., Grant, J., O’Shea, M. & Benjamin, P. R. Modulation of serotonergic neurotransmission by nitric oxide. J. Neurophysiol. 97, 1088–1099 (2007).

12. Yeoman, M. S., Patel, B. A., Arundell, M., Parker, K. & O’Hare, D. Synapse-specific changes in serotonin signalling contribute to age-related changes in the feeding behaviour of the pond snail, Lymnaea. J. Neurochem. 106, 1699–1709 (2008).

13. Kojima, S., Nakamura, H., Nagayama, S., Fujito, Y. & Ito, E. Enhancement of an inhibitory input to the feeding central pattern generator in Lymnaea stagnalis during conditioned taste-aversion learning. Neurosci. Lett. 230, 179–182 (1997).

14. Nakamura, H., Ito, I., Kojima, S., Fujito, Y., Suzuki, H. & Ito, E. Histological characterization of lip and tentacle nerves in Lymnaea stagnalis. Neurosci. Res. 33, 127–136 (1999).

15. Nakamura, H., Kojima, S., Kobayashi, S., Ito, I., Fujito, Y., Suzuki, H. & Ito, E. Physiological characterization of lip and tentacle nerves in Lymnaea stagnalis. Neurosci. Res. 33, 291–298 (1999).

16. Yeoman, M. S., Brierley, M. J. & Benjamin, P. R. Central pattern generator interneurons are targets for the modulatory serotonergic cerebral giant cells in the feeding system of Lymnaea. J. Neurophysiol. 75, 11–23 (1996).

17. Kojima, S., Hosono, T., Fujito, Y. & Ito, E. Optical detection of neuromodulatory effects of conditioned taste aversion in the pond snail Lymnaea stagnalis. J. Neurobiol. 49, 118–121 (2001).

18. Ito, E., Otsuka, E., Hama, N., Aonuma, H., Okada, R., Hatakeyama, D., Fujito, Y. & Kobayashi, S. Memory trace in feeding neural circuitry underlining conditioned taste aversion in Lymnaea. PLoS ONE 7, e43151 (2012).

19. Marra, V., Kemenes, I., Vavoulis, D., Feng, J., O’Shea, M. & Benjamin, P. R. Role of tonic inhibition in associative reward conditioning in Lymnaea. Front. Behav. Neurosci. 4, 161 (2010).

20. Kandel, E. R. The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. Mol. Brain 5, 12 (2014).

21. Xia, Z. & Storm, D. R. Role of signal transduction crosstalk between adenylyl cyclase and MAP kinase in hippocampus-dependent memory. Learn. Mem. 19, 369–374 (2012).

22. Nakamura, H., Kobayashi, S., Kojima, S., Urano, A. & Ito, E. PKA-dependent regulation of synaptic enhancement between a buccal motor neuron and its regulatory interneuron in Lymnaea stagnalis. Zool. Sci. 16, 387–394 (1999).

23. Sadamoto, H., Sato, H., Kobayashi, S., Murakami, J., Aonuma, H., Ando, H., Fujito, Y., Hamano, K., Awaji, M., Lukowiak, K., Urano, A. & Ito, E. CREB in the pond snail Lymnaea stagnalis. cloning, gene expression and function in identifiable neurons of the central nervous system. J. Neurobiol. 58, 455–466 (2004).

24. Sadamoto, H., Yamanaka, M., Hatakeyama, D., Nakamura, H., Kojima, S., Yamashita, M. & Ito, E. Developmental study of anatomical substrate for conditioned taste aversion in Lymnaea stagnalis. Zool. Sci. 17, 141–148 (2000).

25. Straub, V. A., Staras, K., Kemenes, G. & Benjamin, P. R. Endogenous and network properties of Lymnaea feeding central pattern generator interneurons. J. Neurophysiol. 88, 1569–1583 (2002).

26. Scholz, K. P. & Byrne, J. H. Intracellular injection of CAMP induces a long-term reduction of neuronal K+ currents. Science 240, 1666–1666 (1988).

27. Kawai, R., Kobayashi, S., Fujito, Y. & Ito, E. Multiple subtypes of serotonin receptors in the feeding circuit of a pond snail, Zool. Sci. 28, 517–525 (2011).

28. Benjamin, P. R., Staras, K. & Kemenes, G. A systems approach to the cellular analysis of associative learning in the pond snail Lymnaea. Learn. Mem. 7, 124–131 (2000).

29. Azami, S., Wagatsuma, A., Sadamoto, H., Hatakeyama, D., Usami, T., Fujie, M., Koyanagi, R., Azumi, K., Fujito, Y., Lukowiak, K. & Ito, E. Altered gene activity correlated with long-term memory formation of conditioned taste aversion in Lymnaea. J. Neurosci. Res. 84, 1610–1620 (2006).

30. Murakami, J., Okada, R. S., Kobayashi, S., Kobayashi, S., Mita, K., Sakamoto, Y., Yamagishi, M., Hatakeyama, D., Otsuka, E., Okuta, A., Sunada, H., Takigami, S., Sakakibara, M., Fujito, Y., Awaji, M., Moriyama, S., Lukowiak, K. & Ito, E.
Involvement of insulin-like peptide in long-term synaptic plasticity and long-term memory of the pond snail *Lymnaea stagnalis*. *J. Neurosci.* **33**, 371–383 (2013).

31. Mita, K., Okuta, A., Okada, R., Hatakeyama, D., Otsuka, E., Yamagishi, M., Morikawa, M., Naganuma, Y., Fujito, Y., Dyakonova, V., Lukowiak, K. & Ito, E. What are the elements of motivation for acquisition of conditioned taste aversion? *Neurobiol. Learn. Mem.* in press (2013).

32. Murakami, J., Okada, R., Fujito, Y., Sakakibara, M., Lukowiak, K. & Ito, E. Paired pulse ratio analysis of insulin-induced synaptic plasticity in the snail brain. *J. Exp. Biol.* **216**, 1771–1773 (2013).

33. Hatakeyama, D., Okuta, A., Otsuka, E., Lukowiak, K. & Ito, E. Consolidation of long-term memory by insulin in *Lymnaea* is not brought about by changing the number of insulin receptors. *Commun. Integr. Biol.* **6**, e23955 (2013).

34. Sadamoto, H., Kitahashi, T., Fujito, Y. & Ito, E. Learning-dependent gene expression of CREB1 isoforms in the molluscan brain. *Front. Behav. Neurosci.* **4**, 25 (2010).

35. Sadamoto, H., Saito, H., Muto, K., Kinjo, M. & Ito, E. Direct observation of dimerization between different CREB1 isoforms in a living cell. *PLoS ONE* **6**, e20285 (2011).

36. Hatakeyama, D., Fujito, Y., Sakakibara, M. & Ito, E. Expression and distribution of transcription factor CCAAT/enhancer-binding protein in the central nervous system of *Lymnaea stagnalis*. *Cell Tissue Res.* **318**, 631–641 (2004).

37. Hatakeyama, D., Sadamoto, H., Watanabe, T., Wagatsuma, A., Kobayashi, S., Fujito, Y., Yamashita, M., Sakakibara, M., Kemenes, G. & Ito, E. Requirement of new protein synthesis of a transcription factor for memory consolidation: Paradoxical changes in mRNA and protein levels of C/EBP. *J. Mol. Biol.* **356**, 569–577 (2006).

38. Wagatsuma, A., Sadamoto, H., Kitahashi, T., Lukowiak, K., Urano, A. & Ito, E. Determination of the exact copy numbers of particular mRNAs in a single cell by quantitative real-time RT-PCR. *J. Exp. Biol.* **208**, 2389–2398 (2005).

39. Wagatsuma, A., Azami, S., Sakura, M., Hatakeyama, D., Aonuma, H. & Ito, E. De novo synthesis of CREB in a presynaptic neuron is required for synaptic enhancement involved in memory consolidation. *J. Neurosci. Res.* **84**, 954–960 (2006).

40. Vavoulis, D. V., Nikitin, E. S., Kemenes, I., Marra, V., Feng, J., Benjamin, P. R. & Kemenes, G. Balanced plasticity and stability of the electrical properties of a molluscan modulatory interneuron after classical conditioning: a computational study. *Front. Behav. Neurosci.* **4**, 19 (2010).