The Soma of RPeD1 Must Be Present for Long-Term Memory Formation of Associative Learning in *Lymnaea*

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Scheibenstock, Andi, Darin Krygier, Zara Haque, Naweed Syed, and Ken Lukowiak. The Soma of RPeD1 must be present for long-term memory formation of associative learning in *Lymnaea*. *J Neurophysiol* 88: 1584–1591, 2002; 10.1152/jn.00258.2002. The cellular basis of long-term memory (LTM) storage is not completely known. We have developed a preparation where we are able to specify that a single identified neuron, Right Pedal Dorsal 1 (RPeD1), is a site of LTM formation of associative learning in the pond snail, *Lymnaea stagnalis*. We demonstrated this by ablating the soma of the neuron but leaving behind its functional primary neurite, as evidenced by electrophysiological and behavioral analyses. The soma-less RPeD1 neurite continues to be a necessary participant in the mediation of aerial respiratory behavior, associative learning, and intermediate-term memory (ITM); however, LTM cannot be formed. However, if RPeD1’s soma is ablated after LTM consolidation has occurred, LTM can still be accessed. Thus the soma of RPeD1 is a site of LTM formation.

**INTRODUCTION**

We are attempting to discover neuronal sites and causal mechanisms of long-term memory (LTM) formation and storage of an associatively learned behavior within the CNS of *Lymnaea*. Therefore we asked whether LTM could be formed after the soma of one of the neurons, which mediates aerial respiration—the behavior operantly conditioned, was removed. These experiments can be performed because of a number of unique advantages of the *Lymnaea* model system. The first of these is that an identified three-neuron network is both sufficient and necessary to mediate aerial respiratory behavior in *Lymnaea* (Syed and Winlow 1991; Syed et al. 1990, 1992). Since we are examining a form of nondeclarative memory, there is a high probability that the memory of the learned event is encoded with the neuronal circuit that mediates the conditioned behavior (Milner et al. 1998). The second advantage is that aerial respiratory behavior in *Lymnaea* can be operantly conditioned, and it exhibits both intermediate (ITM) and LTM (Lukowiak et al. 1996, 1998, 2001; Smyth et al., 2002). Third, while ITM and LTM formation are both dependent on new protein synthesis, only LTM is dependent on altered gene activity and protein synthesis, whereas ITM is dependent on both altered gene activity and protein synthesis (Mauelshagen et al. 1998; McGAugh 2000; Sutton et al. 2001).

Two different forms of memory, each lasting longer than a few minutes have been described, ITM persists for only a few hours, while LTM persists for days, weeks, or years (Rosenzweig 1998; Rosenzweig et al. 1993). Both ITM and LTM have been demonstrated in *Lymnaea* as a result of different training procedures (Lukowiak et al. 2000). Additionally, ITM has been hypothesized to be dependent only on posttranscriptional protein synthesis, whereas LTM is dependent on both altered gene activity and protein synthesis (Mauelshagen et al. 1998; McGAugh 2000; Sutton et al. 2001).

In molluscan, as well as in crustacean and insect preparations, removal of the soma does not necessarily “kill” the neuron (Parnas et al. 1991). Molluscan neurons are unipolar and the primary neurite is where the majority of synaptic interactions occur (Bullock and Horridge 1965; Kandel 1976). The isolated primary neurite has the ability to survive and function for long periods of time in the otherwise intact animal. Furthermore, the isolated primary neurites (i.e., without the soma) of *Lymnaea* are capable of de novo protein synthesis of injected novel mRNA, and the newly synthesized protein can be functionally integrated into the membrane (Spencer et al. 2000; Van Minnen et al. 1997). We therefore performed ex-

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periments to determine whether selective ablation of RPeD1’s soma (i.e., leaving intact its primary neurite) would result in an inability to encode or access LTM of the operantly conditioned aerial respiratory behavior.

METH ODS

Lymnaea stagnalis, the animal model we used for all of our experiments, was bred and raised in the snail facility at the University of Calgary. All snails used (2.5–3.0 cm) were maintained at room temperature and had continuous access to lettuce in their home eumoxic aquaria.

AssOCIATIVE TRAINING PROCEDURE

OPERANT TRAINING, MEMORY TESTING, AND CHANGE OF CONTEXT PROCEDURES. Individually labeled snails were placed in a 1-l beaker containing 500 ml of water made hypoxic by bubbling N₂ through it 20 min prior to and during training. This is the “standard” hypoxic training procedure. Another training procedure is also used in the studies reported here and is referred to as the “carrot-context.” To create the carrot context, N₂ was first bubbled through an Erlenmeyer flask with chopped carrots and water before being bubbled into the training beaker (see Haney and Lukowiak 2001 for complete details). When we operantly condition Lymnaea, they learn and remember not to perform aerial respiration in the specific context in which they were trained in. Thus Lymnaea trained in the “standard” context behave as naïve snails when placed in the “carrot” context and vice versa. Placing trained snails in a new context can serve as a control for lack of responsiveness or injury. The term “change of context test” means that snails were tested in the context that they were not trained in. This test is used as a control to show that following RPeD1 soma ablation, snails are still as responsive as they were in the initial training session.

In all experiments, the snails were first given a 10-min acclimatization period, where they could perform aerial respiration without receiving any reinforcement. The onset of operant conditioning training was initiated by gently pushing the snails beneath the water surface. In between the training sessions, snails were placed in eu-moxic water where they were allowed to freely perform aerial respiration.

TACTILE STIMULATION OF THE PNEUMOSTOME. In all of the training, memory-test, and change of context test sessions, a gentle tactile stimulus (a sharpened wooden applicator was used) was applied to the pneumostome area (the respiratory orifice) every time the snail began to open its pneumostome to perform aerial respiration. This tactile stimulus only evoked pneumostome-closure; it did not cause the animal to withdraw its foot and mantle into the shell (i.e., the whole-animal withdrawal response). The reinforcing stimulus also did not cause the snails to sink to the bottom of the beaker. The time of each attempted opening was recorded and tabulated.

ASSOCIATIVE TRAINING PROCEDURE. In the operant conditioning training protocol used in the experiments reported here, snails received two 45-min training sessions separated by a 1-h rest interval. A 45-min memory test (MT) session was given to the snails 2 days later. In the data presented in Fig. 5, 1 h after the MT, a 45-min change of context test was also used.

YOKED CONTROL EXPERIMENTS. To show that the changes in behavior resulting from the operant conditioning training procedure are due to associative processes, we performed yoked control experiments, as previously described (Lukowiak et al. 1996, 2000; Spencer et al. 1999). Briefly, yoked animals received a tactile stimulus to their pneumostome area whenever the animal to which they were yoked attempted to open its pneumostome. That is, there was not a contingency between the yoked animal opening its pneumostome and receiving the tactile stimulus. Yoked control experiments were performed on both naïve “intact” Lymnaea and on RPeD1 soma-ablated snails. In the “MT session” yoked control snails did receive the tactile stimulus to their pneumostome when the pneumostome began to open. The number of attempted pneumostome openings in the MT session was compared with the number of attempted openings in the MT session of operantly trained snails and to the first training session of the operantly conditioned snails. As expected, the number of attempted pneumostome openings in the MT session of the yoked control snails was significantly greater than the number of attempted openings in the MT session of the operantly conditioned group; however, it was not significantly different from the number of attempted openings in the first training session of the operantly conditioned group.

BREATHING BEHAVIOR OBSERVATIONS. Snails were placed in a 1-l beaker filled with 500 ml of water made hypoxic by bubbling N₂ through it 20 min prior to and during observations. Animals were allowed a 10-min acclimatization period and then they were gently poked under the water to signify the beginning of the observation period. Total breathing time and the number of pneumostome openings were measured during a 30-min period. Following RPeD1 soma ablation (see Soma ablation) and a 2-day recovery period, the snails’ breathing behavior was tested in a similar manner as before the soma ablation. These data are shown in Fig. 1.

FIG. 1. Right Pedal Dorsal 1 (RPeD1) soma ablation does not alter aerial respiratory behavior. A naïve group of 7 snails were placed in hypoxic pond-water. Following a 10-min acclimatization period, the number of times (A) the snails opened their pneumostomes to perform aerial respiration and the total breathing time (B; duration of each opening × number of openings) were calculated before and 2 days after ablation of RPeD1’s soma. No changes (P > 0.05) in either parameter of aerial respiratory behavior were seen following the surgical procedure and the soma ablation.
Soma ablation

The ablation involved anesthetizing the animals with 1–3 ml of 50 mM MgCl₂ that was injected through the foot. This paralyzed the snail, allowing a dorsal midline incision to be made that exposed the animal’s brain. Using a fine glass electrode, the RPeD1 soma was ablated with gently “poking” it. The incision was small enough to allow the animal to heal without suturing. Animals began to wake from the effects of the anesthetic within several hours of the surgery.

Another group of animals had the soma of LPeD1 (a neuron of similar size to RPeD1), an interneuron that plays no role in aerial respiration, ablated. The exact same procedure for the RPeD1 ablation was used except that LPeD1’s (the “control neuron”) soma was ablated. In both groups of animals, training and testing procedures on snails that were able to perform aerial respiration (~80% of operated snails) were initiated 2 days after surgery. To ensure that the proper cell’s soma had indeed been ablated, a trained individual who was unfamiliar with the experiments attempted to visualize the cells that were ablated under the microscope at the conclusion of the experiment. In all cases the cell that had been ablated could not be found.

Pronase injection

Animals were anesthetized as described above for the soma ablation experiments and a similar dissection followed. Using a micromanipulator, RPeD1 or LPeD1 (the control neuron) was impaled, and pronase (0.6%) was pressure injected into the soma. This procedure has been previously used in the leech to specifically kill single, identified neurons (Bowling et al. 1978; Sahley et al. 1994). Starting 2 days later, animals were placed in the hypoxic training apparatus and their ability to perform aerial respiratory behavior was visually accessed.

Intracellular recording from soma-ablated RPeD1’s neurite

The central ring ganglia were removed from anesthetized Lymnaea as previously described (Spencer et al. 1999; Syed et al. 1992). RPeD1 was impaled with an electrode filled with Lucifer yellow (LY), and the dye was injected into it as previously described (Syed et al. 1992). We waited for 15 min after the filling of RPeD1 with LY and then ablated its soma with the electrode we used to fill it. The CNS was then placed in organ culture (see Syed et al. 1992 for full details) for ≥10 days. Using a blue filter on the light source, we impaled the remaining neurite of RPeD1 and one of the pneumostome motor neurons (H, I, J, K cells).

Standard electrophysiological techniques were used for simultaneous intracellular recordings from both RPeD1’s neurite and a pneumostome motor neuron (H, I, J, K cell) (for complete details of the various salines used please see Spencer et al. 1999). The ability of electrical stimulation (1–5 nA, 500 ms to 2.5 s) to trigger action potentials (APs) in the RPeD1 isolated neurite as well as postsynaptic potentials (PSPs) in the pneumostome motor neuron activity was tested (n = 7).

Blind testing of snails

All behavioral observations were performed “blind.” That is, the researcher performing behavioral observations was unaware of the surgical procedures performed on the animals, and the investigator performing the surgery was unaware of whether the animal had been trained or was naive. Only after all the results were tabulated did we know the outcome of the various experiments.

Operational definitions of learning and memory

We used the same criteria to define learning and memory as in previous studies (Lukowiak et al. 1996, 2000; Spencer et al. 1999). Associative learning is defined as a significant effect of training on the number of attempted pneumostome openings (1-way ANOVA, P < 0.05; followed by a post hoc Fisher’s LSD protected t-test, P < 0.05, within each separate group). The number of pneumostome openings in the final training session has to be significantly less than the number of attempted openings in the first session. The criteria for LTM are as follows.

1) The number of attempted pneumostome openings in the MT session is not significantly different from the number of attempted openings in the last training session.

2) The number of attempted openings in the MT session is significantly less than the number of attempted openings in session 1.

Statistics

A paired Student’s t-test was used to compare differences in breathing time and number of pneumostome openings between cohorts of snails tested following the submersion experiments as well as for the yoked-control experiments.

RESULTS

In all preparations in which RPeD1 was injected with pronase (n = 9), aerial respiratory behavior was not observed when tested 2 days later and on subsequent days of observation (≤10 days later, data not shown). As a control, the soma of a similar sized neuron, not involved with the mediation of aerial respiratory behavior, LPeD1 (the control neuron) was injected with pronase. All of these snails (n = 4) exhibited aerial respiratory behavior when tested 2 days after surgery and pronase injection. Thus pronase injection of RPeD1 prevented aerial respiratory behavior from occurring.

Would RPeD1 soma ablated snails (see METHODS) still have the ability to perform aerial respiration? In 15 snails, we measured aerial respiratory behavior in the hypoxic environment before and 2 days after RPeD1 soma ablation (Fig. 1). There were no significant differences in either the number of pneumostome openings or the total amount of time spent performing aerial breathing pre- or post-RPeD1 soma ablation. Thus RPeD1 soma ablation did not significantly alter the ability of the snails to perform normal aerial respiration. All snails that had the control neuron ablated (LPeD1, n = 50 for all experiments) were competent to perform aerial respiration.

Does the primary neurite of soma ablated RPeD1 continue to function? As assessed by the ability of the RPeD1 soma ablated snails to perform aerial respiration, the answer would appear to be yes. However, to directly demonstrate electrophysiologically that the primary neurite of RPeD1 continues to function, we performed a series of experiments (n = 7) in organ culture (see METHODS) in which RPeD1 was first filled with LY and then had the soma ablated (Fig. 2A). In all preparations, when the isolated primary neurite was impaled 10 days later (Fig. 2B), we found it to be functional in that 1) APs occurred spontaneously or could be evoked by the injection of depolarizing current and 2) APs resulted in 1:1 PSPs in RPeD1’s follower neurons. Shown is a typical recording in which induced activity in the isolated primary neurite of RPeD1 elicits 1:1 excitatory PSPs (EPSPs) in a visceral (H, I, J, K) pneumostome motor neuron. The so-called “alphabet” motor neurons innervate the pneumostome area, and their activity causes pneumostome opening and closure (Syed et al. 1991). These data are comparable to data obtained with RPeD1’s soma intact in isolated ganglia preparations. Thus we conclude that the iso-
Next, a cohort of 30 naïve snails was subjected to the RPeD1 soma ablation procedure 2 days before operant conditioning training commenced, while a second cohort of snails (n = 30) had the soma of the control neuron ablated 2 days before the operant training. The RPeD1 soma ablated snails (Fig. 4A) had the ability to associatively learn, but they could not consolidate the learning into memory. That is, there was a significant effect of the two training sessions on the number of attempted pneumostome openings between the first and second training session (ANOVA, \( F(29, 1) = 98.061.5, P < 0.0001 \)), meeting the criterion for learning. However, the number of attempted openings in the MT session was significantly greater than the number of attempted openings in the last training session (\( P < 0.01 \)) but was not significantly different from the number of attempted openings in the initial training session (\( P > 0.05 \)). Thus these snails did not meet the criteria necessary to demonstrate LTM. Possibly, the reason for the lack of memory was the result of the trauma induced by the surgery. However, in snails in which the control neuron soma was ablated, significantly different data were obtained. These snails also exhibited associative learning, but more importantly, they were able to consolidate the learned behavior into LTM that persisted for 2 days (Fig. 4B). The inability of the RPeD1 soma ablated snails to form LTM was also not due to a difference in the number of reinforcing stimuli received during the training process because there was no significant difference between the number of attempted pneumostome openings in session 1 of the RPeD1 and LPeD1 soma ablated snails and session 2, respectively (paired \( t \)-test; \( P > 0.05 \) for both comparisons). Thus the soma of RPeD1 appears to be necessary for the learning to be consolidated into LTM.

As mentioned in the Introduction, LTM is dependent on altered gene activity and new protein synthesis, while ITM is only dependent on new protein synthesis. In addition, isolated primary neurites of Lymnaea are capable of de novo protein synthesis (Spencer et al. 2000; Van Minnen et al. 1997). Thus we hypothesized that RPeD1 soma ablated snails had the capability to form ITM. We used the same operant procedure that results in associative learning and LTM that persists for 2 days. In naïve, unoperated snails (n = 16) two 45-min operant conditioning training sessions with a 1-h interval between sessions is sufficient to produce learning and LTM that persists for 48 h (Fig. 3). Yoked control snails subjected to this yoked control training procedure (see Methods, n = 12) did not show any memory. That is, when we tested the yoked control snails (i.e., the MT session), we found that the number of attempted pneumostome openings (8.1 ± 1.1) was significantly different (\( P < 0.01 \)) from the number of attempted pneumostome openings (3.2 ± 0.3) in the MT session of the operantly conditioned snails. Moreover, the number of attempted openings in the yoked control snails in the MT session was not significantly different (\( P > 0.05 \)) from the number of attempted openings (8.3 ± 0.5) in the first session of the operantly conditioned snails.

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** Surviving primary neurite of RPeD1 is electrophysiologically competent 10 days after soma ablation. Top: micrograph of primary neurite of RPeD1 that had been filled with Lucifer yellow (LY) dye 10 days previously. After the soma of RPeD1 had been impaled with the LY electrode and dye injected into the neuron, the soma of RPeD1 was ablated with the LY electrode. Central ring ganglia remained in organ culture for 10 days before the surviving primary neurite was re-impaled (bottom). Remains of the LY that filled the soma can still be observed as well as the projections of RPeD1 throughout the CNS and out to the periphery to the pneumostome area. The electrophysiological recording shown in the bottom panel is from the surviving neurite of RPeD1 and a H, I, J, K pneumostome motor neuron. These data were obtained 10 days after RPeD1’s soma was ablated. As can be seen, action potentials (APs) evoked in RPeD1 elicit 1:1 excitatory postsynaptic potentials (EPSPs) in the follower motor neuron. Scale: top, 100 μM; bottom, 3 mV, 10 mV, 500 ms.

![Figure 3](http://jn.physiology.org/)

**FIG. 3.** Associative learning and long-term memory (LTM) formation in *Lymnaea*. A cohort of naïve snails (n = 16) received two 45-min operant conditioning training sessions, separated by a 1-h interval. Two days later they were tested for memory. Learning occurred [ANOVA; \( F(15, 1) = 121.5, P < 0.0001 \)] as did consolidation of the learning into LTM [session 3 was not significantly different from session 2 (\( P > 0.05 \)) and session 3 was significantly different from session 1 (\( P < 0.01 \))]. Yoked control animals did not learn and thus did not form LTM.
Thus the criteria for memory were met in these controls.

conditioning training procedure shown in Fig. 3, but tested for memory 2 h after the last training session (n = 12, Fig. 5). As can be seen, ITM was demonstrated. That is, the number of attempted pneumostome openings in the MT session was not significantly different (P > 0.05) from the number of attempted openings in the last training session (session 2) but was significantly different from the number of attempted openings in session 1 (P < 0.01). Thus RPeD1 soma-ablated snails are competent to associatively learn and form ITM that persists for 2 h.

It was possible that the soma of RPeD1 was not needed for the consolidation process leading to the formation of LTM but only for its storage or recall. Thus a final experiment was performed to show that the soma of RPeD1 was required for the formation of LTM (Fig. 6). We first trained naïve, unoperated snails (n = 20) using the same training procedure as in Fig. 4. Two hours after the last training session, the soma of RPeD1 was ablated. Two days later we tested to see if LTM was present, and it was. The number of attempted pneumostome openings in the MT session was not significantly different from the last training session (session 2; P > 0.05) but was significantly different from the first training session (P < 0.01).
To demonstrate that the significantly reduced number of attempted pneumostome openings in the MT session was a reflection of memory and not the result of surgical trauma, we challenged these snails 1 h later with a different context test (see METHODS). The number of attempted pneumostome openings in the “carrot-odor” context was significantly different from the number of openings in the memory test ($P < 0.001$) and was not different from the number of attempted openings in the first training session ($P > 0.05$). That is, these snails were capable of performing aerial respiratory behavior as well as they did before the RPeD1’s soma was removed. Two conclusions can be drawn from these data: 1) RPeD1 soma is necessary for the processes that lead to LTM consolidation and 2) the soma of RPeD1 is not necessary to access an already encoded LTM. Finally, in a cohort of snails ($n = 12$) subjected to the yoked control procedure, we also removed RPeD1’s soma 2 h after the last training session. When we tested these snails 2 days later we found that the number of attempted pneumostome openings in the MT session ($7.8 \pm 0.9$) was significantly different ($P < 0.01$) from the number of attempted pneumostome openings ($4.1 \pm 0.3$) in the MT session of the operantly conditioned snails. Thus removal of RPeD1’s soma after the yoked control procedure yields a significantly different result from that seen with snails trained using the operant conditioning procedure.

**DISCUSSION**

Our data show that the presence of RPeD1’s soma is necessary for the consolidation of associative learning into LTM. The presence of RPeD1’s soma is, however, not necessary for aerial respiratory behavior, the electrophysiological functioning of the primary neurite, associative learning, the formation of ITM, or the recall of a previously encoded LTM. These are all novel and significant findings. In the past, small brain regions in both vertebrates and invertebrates have been shown to be necessary for the storage of memory (Kandel and Pittenger 1999; Pascual and Prétat 2001). For example, in the *rut* mutant of *Drosophila*, Zars et al. (2000) showed that the Kenyon cells of the mushroom body are necessary for the storage of a short-term memory. They did this by restoring the *rut* adenylyl cyclase activity in only the Kenyon cells of the mutant *Drosophila* brain. Without the restoration of the specific enzyme activity (i.e., the mutation), flies did not exhibit short-term memory. Thus they concluded that the memory trace for the learned behavior was restricted to the Kenyon cells. More recently, Pascual and Prétat (2001) demonstrated that specific lobes, the vertical lobes, in the mushroom body were necessary for LTM. In mammals, including humans, using noninvasive imaging techniques, specific surgical interventions, and “opportunistic” traumatic clinical events, specific regions of the brain have been shown to be necessary for the formation and/or storage of specific long-lasting memories (Milner et al. 1998; Schacter 2001) Here we show that a single neuronal cell body is a necessary site for the formation of LTM.

A “functional” RPeD1 is necessary for aerial respiratory behavior, but functionality does not require the presence of the soma of RPeD1. Killing the neuron by pronase injection, as opposed to just ablating the soma, abolishes this behavior. Similar conclusions using pronase injection techniques have been made regarding the necessity of an identified neuron for a specific behavior in the leech (Bowling et al. 1978; Sahley et al. 1994). Our present findings are also consistent with the data from experiments where the CPG that controls aerial respiratory behavior was reconstructed in culture (Syed et al. 1990). RPeD1’s presence was necessary to initiate and maintain rhythmogenesis. The data presented here also complement our earlier study where we showed that VD4, also one of the three CPG neurons, was necessary for the aerial respiratory behavior (Syed et al. 1992). However, the presence of RPeD1’s soma is not necessary for aerial respiration, because the surviving neurite, where the necessary synaptic interactions occur, is functional and thus sufficient to play its role in the mediation of aerial respiratory behavior. Aerial respiratory behavior, as evidenced by number of openings and total breathing time, is not significantly different following the ablation of RPeD1’s soma (Fig. 1). In addition, the direct electrophysiological data show that the isolated (i.e., without the soma) RPeD1 neurite can survive for $\geq 10$ days in organ culture and is still competent to produce APs and to elicit 1:1 PSPs in follower motor neurons.

We chose to perform the soma ablation experiments on RPeD1 to see if it was a site of LTM formation primarily because neural correlates of associative learning and LTM had been demonstrated in RPeD1 (Spencer et al. 1999), and experimentally it is relatively easy to ablate RPeD1’s soma without destroying its neurite. As well, since we are studying nondeclarative memory, there was a high probability that memory would be encoded within members of the neuronal circuit that mediate the behavior (Milner et al. 1998). RPeD1 soma-less snails are capable of associative learning and encoding a memory that persists for $\leq 2$ h (i.e., ITM). ITM in *Lymnaea* (Smyth et al. 2002) and in other systems, while dependent on new protein synthesis, does not require altered gene activity (Crow et al. 1999; Rosenzweig et al. 1993; Sutton et al. 2001). Thus ITM appears to be encoded by already present mRNAs. Molluscan isolated primary neurites (i.e., soma-less) have the capability both for de novo protein synthesis (Martin et al. 1997; van Minnen et al. 1997) and the functional integration of the newly synthesized product into the membrane of the isolated neurite (Spencer et al. 2000). We therefore expected and subsequently found that RPeD1 soma-less snails could exhibit ITM.

In every organism so far studied and in our own preliminary data (Sangha et al. 2001), LTM has been shown to be dependent on both altered gene activity and new protein synthesis (Rosenzweig 1998). We made use of this fact and the ability of molluscan primary neurites to continue to function without their somata for up to 4 wk in intact snails, to devise experiments to show that an identified neuron was a site of LTM formation. Our working hypothesis was that RPeD1 played a necessary role in the encoding of LTM. We therefore expected that associative learning could not be consolidated into LTM following removal of RPeD1’s soma because the surviving neurite cannot receive any new mRNA or proteins from its former nucleus. We ruled out the possibility that the failure of LTM formation in the RPeD1 soma ablated snails was the result of surgical procedures per se. That is, in experiments where the soma of the control neuron was ablated, LTM was still observed. In addition, both learning and ITM were shown...
in RPeD1 soma-less snails, indicating that learned behavior could be remembered for a few hours. However, in snails without RPeD1’s soma, the consolidation process by which the associative learning is encoded into LTM does not occur (Fig. 4A). We can thus conclude that RPeD1 is a site for LTM formation.

It was possible that in RPeD1 soma ablated snails LTM was encoded, but either could not be assessed or retrieved without the soma. To rule out this possibility, we ablated the soma of RPeD1 in snails that had been operantly conditioned (Fig. 6). We found that these snails still possessed memory. As an additional control to show that these snails were not unresponsive as a result of the surgery after the operant conditioning had occurred, we challenged these snails with a “different-context” test. The snails responded as they did in the initial training session (when the soma of RPeD1 was present). Thus RPeD1 soma ablated snails still had the ability to assess or retrieve a previously encoded memory. Finally yoked control snails in which RPeD1’s soma was ablated 2 h after the last training session showed no evidence of memory or a behavioral phenotype similar to memory.

We do not know yet what physically constitutes LTM in the Lymnaea model system. However, we now know that once established, the changes that constitute LTM do not require the soma of RPeD1 to be present. The functional surviving primary neurite possesses the necessary molecular machinery to maintain the changes that constitute LTM. How this occurs we do not know. Previously it has been suggested that the ability of an isolated invertebrate neurite to survive for long periods of time may depend on the “donation” of a molecular message from surrounding, supportive glia (Parnas et al. 1991). Whether this is correct in our case needs to be tested.

Having established that RPeD1 is a site for LTM storage, we are now in a position where we can begin experiments to determine what these changes are. We may now be able to directly ask in an identified single neuron which genes are turned on or off, what gene products constitute the physical basis of memory storage, and where are the sites within the cell where these proteins are located. Finally, we must point out that although we have shown that RPeD1’s soma is necessary for LTM, we have not shown that changes in it are sufficient for LTM. It may be possible to directly determine if RPeD1’s soma is both necessary and sufficient for LTM by transplantation experiments (see Syed et al. 1992). If this was the case, then transplantation of an “educated” RPeD1 to a naive snail would result in the recipient snail exhibiting the behavioral phenotype of a trained snail with LTM. However, our working hypothesis is that altered gene activity must occur in all of the CPG neurons for memory consolidation, and thus we are not overly confident that the transplantation of a single cell will result in the transfer of memory.

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