The Journal of Experimental Biology 215, 536-542
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doi:10.1242/jeb.061432

Sensory input from the osphradium modulates the response to memory-enhancing stressors in *Lymnaea stagnalis*

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Accepted 15 September 2011

SUMMARY

In the freshwater environment species often rely on chemosensory information to modulate behavior. The pond snail, *Lymnaea stagnalis*, is a model species used to characterize the causal mechanisms of long-term memory (LTM) formation. Chemical stressors including crayfish kairomones and KCl enhance LTM formation (≥24 h) in *Lymnaea*; however, how these stressors are sensed and the mechanism by which they affect the electrophysiological properties of neurons necessary for memory formation are poorly understood. Here, we assessed whether the osphradium, a primary chemosensory organ in *Lymnaea*, modulates LTM enhancement. To test this we severed the osphradial nerve proximal to the osphradium, using sham-operated animals as controls, and assessed the behavioral and electrophysiological response to crayfish kairomones and KCl. We operantly conditioned aerial respiratory behavior in intact, sham and osphradially cut animals, and tested for enhanced memory formation after exposure to the chemical stressors. Sham-operated animals displayed the same memory enhancement as intact animals but snails with a severed osphradial nerve did not show LTM enhancement. Extracellular recordings made from the osphradial nerve demonstrate that these stressors evoked afferent sensory activity. Intracellular recordings from right pedal dorsal 1 (RPeD1), a neuron necessary for LTM formation, demonstrate that its electrophysiological activity is altered by input from the osphradium following exposure to crayfish kairomones or KCl in sham and intact animals but no response is seen in RPeD1 in osphradially cut animals. Therefore, sensory input from the osphradium is necessary for LTM enhancement following exposure to these chemical stressors.

Key words: *Lymnaea*, long-term memory, osphradium, behavioral ecology.

INTRODUCTION

The ability to form memory is an important adaptive trait: it can help the animal remember areas high in resources, and it can also help the animal avoid areas of danger. It is well known that stress can modulate memory formation, either by enhancing or blocking memory depending on the nature of the stress and when it is experienced relative to the learning event (Shors, 2004; Kim and Diamond, 2002). The formation and retrieval of memory are dynamic processes, and can be modulated by stress and associated traumatic events (Gordon and Spear, 1973; Kim and Diamond, 2002; Cahill et al., 2003). Stress during learning is commonly associated with negative effects on memory retrieval (Kim et al., 2005) but certain relevant biological stressors can be effective in enhancing memory (Cahill et al., 2003).

How stress alters the molecular and electrophysiological properties of neurons that are necessary for the formation of behavioral long-term memory (LTM) has not been fully elucidated, in part because of the complexity of the mammalian brain. For example, certain acute stressors (e.g. electric shock) alter the formation of long-term potentiation (LTP) in the CA1 region of the hippocampus (Shors et al., 1989), whilst other stressors enhance long-term depression (LTD) in the hippocampus (Yang et al., 2005). However, it is not clear what the mechanism(s) is as to how stress alters synaptic plasticity thought to underlie behavioral LTM (Howland and Wang, 2008). In the rodent brain it is often thought that the effects of acute stress on learning and memory are the result of increased levels of corticosterone (de Quervain et al., 1998). However, this hormone can be increased to similar levels by exposing rats to either a cat or a sexually receptive female rat but only those rats exposed to the cat showed blockage of hippocampal-dependent LTM (Woodson et al., 2003). In an attempt to overcome some of the complexities of using mammalian preparations we have utilized our *Lymnaea* model system where it is possible to study at the single neuron level how stress alters the activity of a neuron necessary for behavioral LTM.

Similarly to other animals tested, stress experienced before, during or after a learning event can alter the ability of *Lymnaea* to form memory, with the nature of the stressor having either neutral, negative or positive effects on memory formation (Lukowiak et al., 2008; Lukowiak et al., 2010). As Kim and Diamond have pointed out, for a given stimulus to be considered as a 'stressor', a number of criteria have to be met including whether the stimulus is sensed by the organism (Kim and Diamond, 2002). However, it is unclear in the *Lymnaea* model system how the stressors that alter memory formation are sensed by the organism and how this sensory input alters neuronal activity. Here, we attempted to elucidate the role played by a candidate sensory structure, the osphradium, in the modulation of LTM formation at both the behavioral and neurophysiological levels. The osphradium is an external sensory organ situated directly above the pneumostome that *Lymnaea* uses for chemosensation (Wedemeyer and Schild, 1995). It has been previously demonstrated that neurons in the osphradium show an electrophysiological response to a wide range of chemicals, and hence may be a primary method by which the snail senses external stressors (Wedemeyer and Schild, 1995; Kamardin et al., 2001).

However, despite this knowledge, there has been little work demonstrating how the sensory input through the osphradium alters behavior in *Lymnaea*.

Dalesman et al. (Dalesman et al., 2011a) have shown that the osphradium mediates the blocking effect that low environmental Ca^{2+} has on LTM formation whereas there was no effect of severing the osphradial nerve on the response to crowding. Further, Il-Han et al. (Il-Han et al., 2010) demonstrated that osphradial input is necessary for enhanced memory formation in the presence of crayfish kairomones (crayfish effluent, CE), although neither study identified the electrophysiological effects of chemical sensation by the osphradium in the central nervous system (CNS). Here, we investigated the modulation of both behavioral and electrophysiological responses to crayfish kairomones and KCl (25 mmol l^{-1}) *via* input from the osphradium. Both of these stressors enhance LTM formation; however, stressors that induce a similar phenotype need not act through the same sensory system (Dalesman et al., 2011a). While there is evidence that CE enhances memory due to sensory input from the osphradium, we do not have information about how KCl modulates memory enhancement, nor do we know how sensory information alters activity in the CNS. We therefore tested for changes in the electrophysiological properties of right pedal dorsal 1 (RPeD1), as this neuron has also been demonstrated to be necessary for LTM formation following our operant conditioning procedure (Scheibenstock et al., 2002).

MATERIALS AND METHODS

Subjects

Adult *Lymnaea stagnalis* (L.) obtained from a population originating from wild snails collected in the 1950s from canals in a polder near Utrecht (spire height of ~25 mm) were used to perform all experiments. The snails were reared at the University of Calgary's Biological Sciences building, and maintained in artificial pond water (~0.25 g l^{-1} Instant Ocean®, Aquarium Systems Inc., Mentor, OH, USA) with the addition of calcium carbonate to maintain calcium levels of at least 50 mg l^{-1} with additional access to sterilized cuttlefish (*Sepia officinalis*, L.) bone (Hermann et al., 2009). The animals were fed *ad libitum* with lettuce and Aquamax-carnivorous Grower 600 trout pellets (Purina Mills LLC, St Louis, MO, USA). Snails were transferred to the laboratory at least one week before experiments were performed, and maintained in oxygenated artificial pond water (0.26 g l^{-1} Instant Ocean®) containing calcium sulphate dehydrate ([Ca^{2+}] 80 mg l^{-1}) at room temperature (~20°C) under 16 h:8 h light:dark conditions. The snails were kept at a density of one snail per liter, and were fed romaine lettuce *ad libitum*.

Surgical procedure

In these experiments, three groups of animals were used: (1) animals in which the osphradial nerve was severed (cut) proximal to the osphradium; (2) animals that underwent the same surgical procedure minus the severing of the nerve to control for any effects of the procedure and anesthetic (sham); and (3) animals that did not undergo any surgical procedure at all (intact). Cut and sham snails were anesthetized first by using iced pond water, and then injected with 2 ml of 50 mmol l^{-1} $MgCl_2$ *via* the foot into the hemocoel. The magnesium chloride acted as a relaxant, preventing withdrawal into the shell and allowing access to the area around the osphradium. When the anesthetized animals were placed into a dissection dish, a small slit was made in the skin to access the osphradial nerve. In sham animals, the small slit was made but the nerve was left uncut. In the cut animals, however, the nerve was severed proximal to the osphradium. The small cut made to access the osphradial nerve heals very quickly without requiring further intervention. Animals rapidly recovered from both procedures, and were behaving otherwise normally within a few hours. All animals were then allowed a minimum of 72 h of recovery time before any experiments were conducted. All training and testing procedures, both behavioral and electrophysiological (RPeD1), were carried out blind to the surgical procedure the snail had received.

Operant conditioning

Snails were individually labeled at least 24 h in advance. 500 ml of room temperature water in a 1 liter beaker was made hypoxic (<0.1 ml O_2 l^{-1}) by bubbling N_2 through it for at least 20 min. This is the 'standard' training and testing context (Lukowiak et al., 1996; Lukowiak et al., 1998). Snails were then placed into the training beaker and allowed to acclimate to the new environment for 10 min. During this time, the snails could move freely and perform aerial respiration undisturbed. Training was initiated by gently pushing the snails below the surface of the water. In all training sessions (TS) and memory tests (MT) a gentle tactile stimulus was applied to the pneumostome every time the snail initiated aerial respiration by opening the orifice. A sharpened wooden applicator was employed to administer this 'poke'. The 'poke' was of force sufficient to cause the animal to close the orifice but not enough to cause the snail to withdraw into its shell and sink to the bottom of the beaker. All sessions were 30 min long, consisting of a single training session (TS') followed by a MT 24 h later. Typically, if snails are subjected to a single 30 min TS, a memory that persists for only 3 h (termed long–intermediate-term memory, ITM) is observed. ITM requires new protein synthesis. If, however, snails are subjected to two 30 min TS (separated by 1 h), LTM is observed. LTM requires both altered gene activity and new protein synthesis (Sangha et al., 2003). Hence, if there is no memory enhancement occurring, the training regime used here will not result in LTM formation at 24 h. Between TS the snails were returned to eumoxic home aquaria and were freely allowed to perform aerial respiration. Breathing behavior was not monitored while in the home aquaria. All behavioral trials were carried out blind to the surgical procedure that the snail had received.

Application of aversive stimuli

Two types of stressor were used, both of which have been found previously to enhance LTM formation. To evoke an acute stress response in *Lymnaea*, the snails were exposed to 25 mmol l^{-1} KCl (i.e. the KCl bath). Snails that received the KCl bath before training were taken from their home aquarium and placed into an individual 37 mm Petri dish containing 4 ml of the 25 mmol l^{-1} KCl for 30 s. This was enough solution to cover the foot and osphradium of the snail but not enough to submerge it. The snails were then placed in the hypoxic training beaker for acclimatization (10 min), followed by a 30 min training procedure. For the CE experiment, the 30 min TS was performed in pond water containing crayfish kairomones. This water was prepared by leaving a crayfish (6±1 cm mantle length) in standard pond water for a minimum of 1 h. In both cases snails were tested for LTM formation at 24 h in pond water alone.

Extracellular nerve recording

The snails were exposed to a two-bath system using a semi-intact preparation. The semi-intact preparations used were prepared following protocols previously developed in our laboratory (McComb et al., 2005; Orr et al., 2007; Orr and Lukowiak, 2008; Orr et al., 2009). The CNS was kept in snail saline and the body was perfused by pond water. The osphradial nerve was then cut as

close to the right parietal ganglia as possible and teased away from the connective tissue holding it to the body wall. A glass microelectrode was pulled to a resistance of 20–40 MΩ and the tip was broken to match the size of the nerve. The electrode was moved towards the cut end of the nerve and the nerve was then sucked into the electrode. The extracellular signal from the nerve was amplified using an A-M systems Microelectrode AC amplifier, Model 1800 (A-M Systems, Sequim, WA, USA) and recorded and analyzed using Chart 5 (AD Instruments Inc., Colorado Springs, CO, USA).

Exposure of the body (and osphradium) to crayfish kairomones was accomplished by switching the inflow to the dish from pond water to CE. The solution bathing the body was turned over rapidly, as the volume of the body chamber was ~2.5 ml and the flow of the pond water into the dish was 7.5 ml min^{-1}.

Electrophysiological readings

Snails were either left intact, sham-operated or the osphradial nerve was severed at least 72 h prior to recording. Snails were then exposed to a two-bath system using a semi-intact preparation, leaving the head and the body of the animal in a separate compartment from the CNS. This preparation was used to measure RPeD1 activity. The level of the bathing solution (pond water) was such that the pneumostome was right at the surface, because submersion would prevent the process of opening the pneumostome. The preparations were rested for at least 20 min prior to impaling the neurons with a sharp glass microelectrode that had been filled with saturated K_2SO_4 solution (tip resistances ranged from 20 MΩ to 75 MΩ). They were then given another 15 min stabilization period prior to collection of electrophysiological data, to give a total time of 35 min between the dissection procedure and data collection. Intracellular signals were amplified using a Neurodata Instruments IR283 amplifier (New York, NY, USA) and displayed simultaneously on a Macintosh PowerLab/4SP (AD Instruments Inc.) and a Hitachi oscilloscope (Tokyo, Japan). Recordings were stored and analyzed through Chart 5 software (AD Instruments Inc.) using a 600 s trace for data analysis. Electrode balance was measured at both the start and end of each experiment. If the resistance had changed by more than 5%, the trace was discarded.

One of the measurements taken was the number of action potential bursts fired. Previous work in our laboratory has shown that the RPeD1 bursts of action potentials that trigger pneumostome opening have particular properties (Dalesman et al., 2011a). Hence, each burst was defined as a period of sustained depolarization containing 2–20 action potentials, and the burst was not considered over until the membrane potential had returned to its resting state. The second measurement taken was the number of spikes, or individual action potentials, seen in the recording period. These procedures were carried out initially in the absence of a memory-enhancing stimulus (i.e. in pond water alone), following which the body of the animal only was exposed to either a CE bath or 10 drops of KCl were placed directly on top of the pneumostome. This allowed us to directly compare the electrophysiological activity of RPeD1 in the same animal both in the absence of a stimulus and also following exposure of the body to a memory-enhancing stimulus. The CE recordings were taken over a 10 min time period whereas the KCl recordings were taken over a 2 min time period. The KCl was not flushed constantly because it was dropped onto the pneumostome, and hence the effect would be blunted if a 10 min recording was taken. For consistency, the CE recordings were averaged such that the number of bursts and spikes per 2 min time period are shown. The two-bath system ensured that the CNS was in purely a saline solution throughout the experiments. The investigator performing the electrophysiological experiments was ‘blind’ regarding whether the preparation came from a control, cut or sham-operated snail.

Statistical analysis

Behavioral data were analyzed in SPSS 17.0 (SPSS Inc., Chicago, IL, USA); data for each stressor tested were analyzed separately using repeated-measures ANOVA with training period (training *vs* test) as the within-subject factor and surgery (intact, sham or cut) as the between-subject factor. Where overall significance was found, *post hoc* paired *t*-tests were used to assess whether the number of pneumostome opening attempts during the test was significantly lower than that during training (pairwise results are presented in the figures). Electrophysiological data were also analyzed in SPSS Inc. using repeated-measures ANOVA with stress application (present *vs* absent) as the within-subject factor and surgery (intact, sham or cut) as the between-subject factor. *Post hoc* paired *t*-tests were used to assess pairwise differences between the response in pond water and when the stressor was applied. Mauchley’s test was used to confirm sphericity prior to analysis.

RESULTS

Behavioral results

There was no significant difference in the number of attempted pneumostome openings in the first TS under all ‘surgical’ conditions (i.e. cut, sham, intact) for both the CE (N=35) and KCl (N=39) experiments (Tukey’s test: P>0.05 for all pairwise tests). Therefore, the initial number of breathing attempts was not affected by the different surgical procedures. In intact (N=12) and sham-operated (N=12) snails exposed to CE during the single 30 min TS, LTM formation was observed 24 h later. However, in snails whose osphradial nerve had been severed, exposure to CE (N=11) during training did not result in LTM (Fig. 1; repeated-measures ANOVA $F_{2,32}$=18.89, P<0.001). Similarly, following exposure to KCl immediately prior to training, the intact (N=12) and sham (N=13) snails exhibited LTM formation whereas the cut snails (N=14) did not (Fig. 2; repeated-measures ANOVA $F_{2,36}$=11.72, P<0.001).

Electrophysiology results

We now wished to see if the obtained behavioral results would also be reflected in the activity of the osphradial nerve itself, and RPeD1 (the neuron known to be a necessary site of LTM formation).

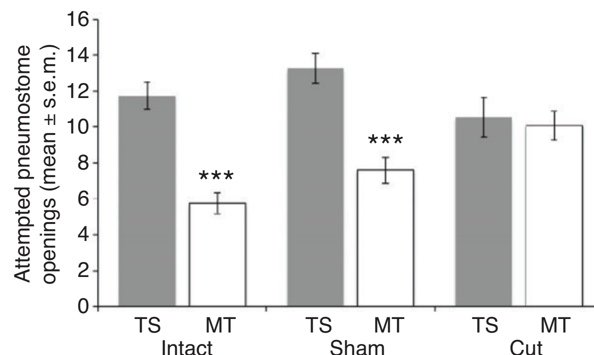

Fig. 1. Enhanced memory formation following exposure to crayfish effluent (CE). The number of attempted pneumostome openings during a single training session (TS) (30 min, gray) conducted in the presence of CE and the test for memory [30 min, memory test (MT), white] 24 h later are shown. Three groups of animals (cut, sham and intact) were used. ***=differs significantly from TS within the specific training group (paired *t*-test, P<0.001).

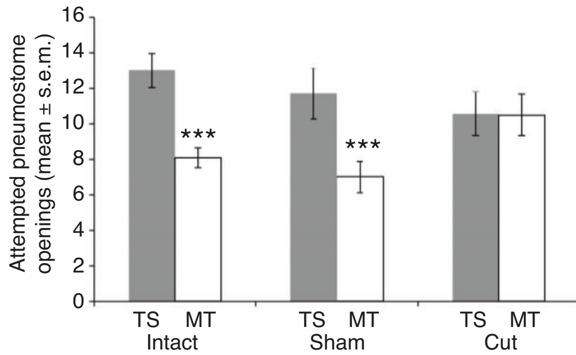

Fig. 2. Enhanced memory formation following exposure to KCl. The number of attempted pneumostome openings during a single training session (TS) (30 min, gray) following a 30 s bath in 4 ml of 25 mmol l^{-1} KCl solution and the test for memory (MT) (30 min, white) 24 h later are shown. Three groups of animals (cut, sham and intact) were used. ***=differs significantly from TS within the specific training group (paired *t*-test, $P<0.001$).

Application of either CE or KCl onto the osphradium resulted in an increase in afferent activity in the osphradial nerve (Fig. 3: recording shown from the CE application only).

Fig. 4A shows the activity of RPeD1 in a representative semi-intact preparation made from a control (i.e. intact animal) with pond water bathing the body (i.e. pneumostome/osphradial area). Bathing the body in CE results in an increase in this activity in both intact (Fig. 4B) and sham-operated (Fig. 4C) animals. However, exposing the body of osphradially cut animals in a CE bath does not elicit any change in electrical activity in RPeD1 (Fig. 4D).

We compared the burst frequency (Fig. 5A) and the number of spikes counted (Fig. 5B) over a 10 min period after exposing the body to CE. There was no statistical difference in the electrophysiological parameters between surgical procedures (intact, sham and cut) when pond water was bathing the periphery (Fig. 5A,B; Tukey's test: $P>0.05$). However, exposing the body to CE significantly increased both burst frequency (Fig. 5A; repeated-measures ANOVA $F_{2,20}$=5.161, P=0.016) and the number of elicited spikes (Fig. 5B; repeated-measures ANOVA $F_{2,20}$=23.654, $P<0.001$) in the intact and sham semi-intact preparations but not in the cut osphradial nerve semi-intact preparations.

Similarly, prior to the application of KCl, there was no statistical difference in the electrophysiological parameters between surgical procedures (intact, sham and cut) when pond water was bathing the

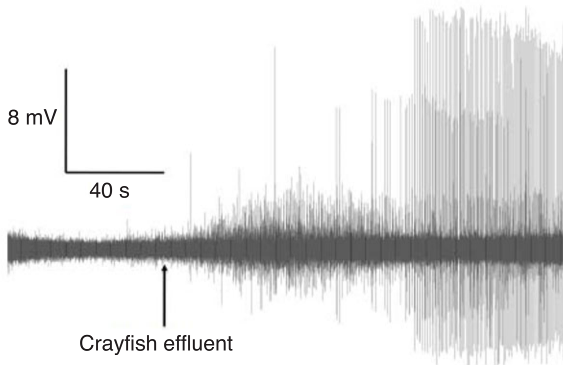

Fig. 3. Extracellular osphradial nerve recordings upon exposure to crayfish effluent (CE) and KCl. Extracellular nerve recording of the osphradial nerve and its response to CE. The recording was taken over a period of 10 min.

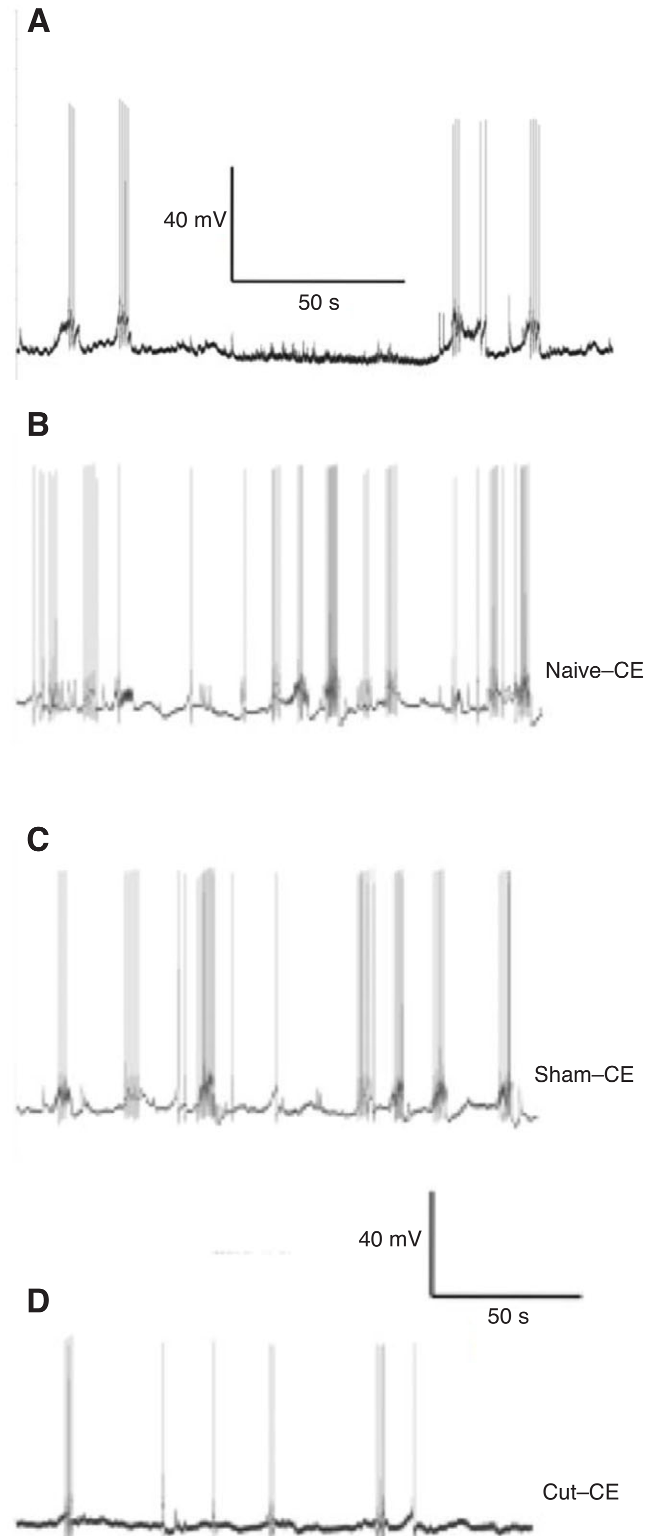

Fig. 4. (A) Firing pattern of right pedal dorsal 1 (RPeD1) under control conditions. A normal trace of bursts from the neuron RPeD1 in an intact animal in standard pond water is shown. No chemical stressors or signals have been added to the bath. A comparison of the electrophysiological response of the neuron RPeD1 between an intact (naïve, B), sham (C) and osphradially cut (D) snail exposed to crayfish effluent (CE) is also presented. Following a 10 min period in pond water, the animals were exposed to a bath containing crayfish kairomones for another 10 min time period.

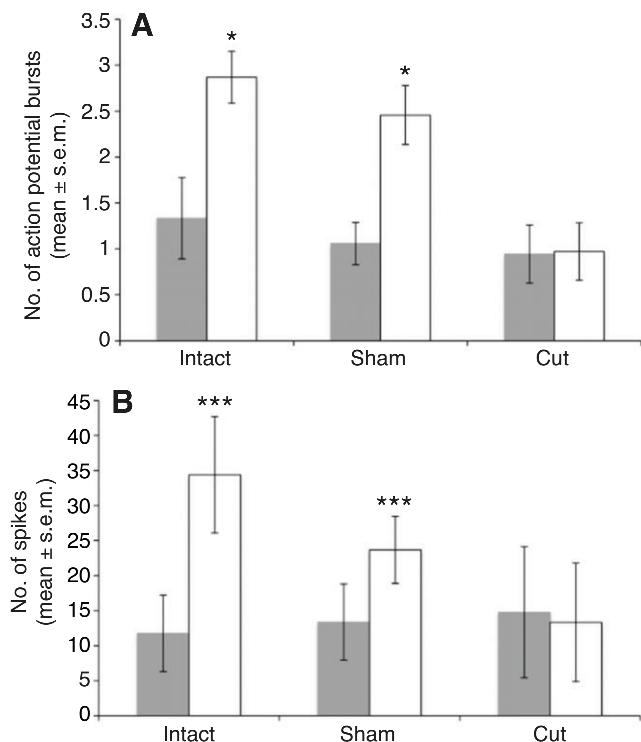

Fig. 5. (A) Comparison of burst frequency in right pedal dorsal 1 (RPeD1) in response to crayfish effluent (CE). The number of bursts of action potentials by RPeD1 in pond water (gray) and following exposure to CE (white) in the same snail are shown. Measurements were taken over a 10 min time period but the values were averaged such that the number of bursts per 2 min time period is presented. *=differs significantly from pond water within the specific training group (paired *t*-test, $P<0.05$). (B) Comparison of action potential frequency in RPeD1 in response to CE. The number of spikes (action potentials) by RPeD1 in pond water (gray) and following exposure to CE (white) in the same snail are shown. Measurements were taken over a 10 min time period but the values were averaged such that the number of action potentials per 2 min time period is presented. N=9, intact; N=7, sham; N=7, cut. ***=differs significantly from pond water within the specific training group (paired *t*-test, $P<0.001$).

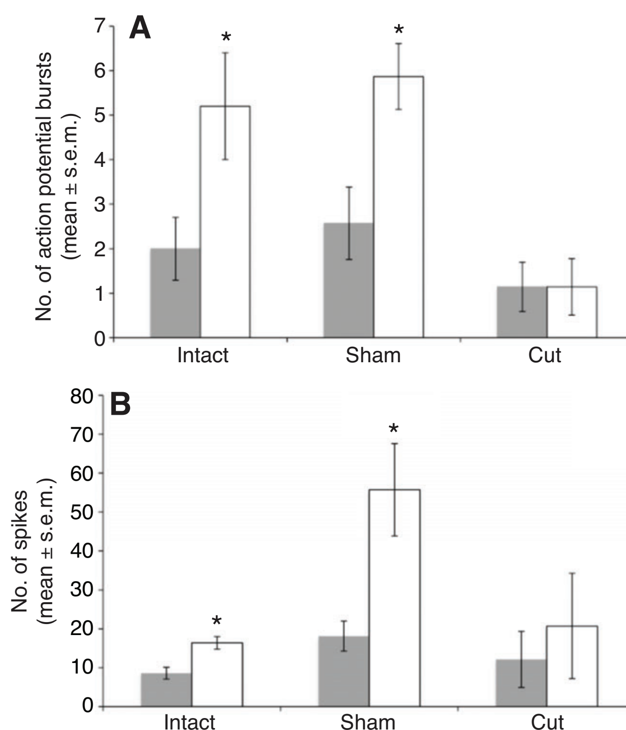

Fig. 6. (A) Comparison of burst frequency in right pedal dorsal 1 (RPeD1) in response to KCl. The number of bursts of action potentials by RPeD1 over a 2 min span in pond water (gray) and following 10 drops of 25 mmol l^{-1} KCl on the pneumostome (KCl, white) of the same snail over a 2 min time period are shown. *=differs significantly from pond water within the specific training group (paired *t*-test, $P<0.05$). (B) Comparison of action potential frequency in RPeD1 in response to KCl. The number of spikes (action potentials) by RPeD1 over a 2 min span in pond water and following 10 drops of 25 mmol l^{-1} KCl on the pneumostome of the same snail over a 2 min time period are shown. N=5, intact; N=7, sham; N=7, cut. *=differs significantly from pond water within the specific training group (paired *t*-test, $P<0.05$).

periphery (Fig. 6A,B; Tukey's test: $P>0.05$). However, when KCl drops were applied to the pneumostome there was a significant increase in burst frequency (Fig. 6A; repeated-measures ANOVA $F_{2,16}=16.81$, $P<0.001$) and the number of spikes (Fig. 6B; repeated-measures ANOVA $F_{2,16}=5.49$, $P=0.015$) in the intact and sham semi-intact preparations but, once again, not in the cut osphradial nerve semi-intact preparations.

DISCUSSION

It is quite evident based on our own life experiences that stress affects how we learn and form memory. Some stressors block or make memory more difficult to form while other stressors result in enhanced memory (Lukowiak et al., 2010). In humans, stressful stimuli in some cases results in a psychopathological state. One example is post-traumatic stress disorder (PTSD), in which an event cannot be forgotten (Mahan and Ressler, 2011). However, the causative mechanisms by which stressors alter memory formation at the neuronal level are not clear (Howland and Wang, 2008; Bangasser and Shors, 2010). We have taken advantage of some of the unique attributes of our *Lymnaea* model system to begin to elucidate the causal mechanisms by which stressors alter memory formation.

Our data demonstrate that two memory-enhancing stressors, CE and KCl, are detected by the osphradium, an external sensory organ. Detection of these stressors causes an increase in afferent activity of the osphradial nerve connecting this organ to the CNS. This afferent sensory information significantly reduces the excitability of RPeD1 so that the neuron is 'primed' to form LTM. The result of this change in electrical activity is that the snail now forms LTM more easily than in control conditions, i.e. LTM formation is enhanced. When the osphradial nerve is severed both the reduction in electrophysiological activity in RPeD1 and enhancement of memory is prevented in the presence of CE and KCl, confirming that input from the osphradium is necessary for memory enhancement.

The osphradium is innervated by a small nerve, which we and others (e.g. Wedemeyer and Schild; 1995; Kamardin et al., 2001) have called the osphradial nerve. This small nerve branches off the larger right internal pallial nerve, which exits from the right parietal ganglion (Bullock and Horridge, 1965). Fortunately, the osphradial nerve courses close to the skin and can be easily severed without impacting the viability of the snail. Thus, cutting the osphradial nerve does not alter the snail's normal aerial respiratory behavior (Il-Han et al., 2010) or the snail's ability to learn and form memory following

a more robust training regime (Dalesman et al., 2011b). The modulatory effects of stressors could potentially be due to altering breathing rate directly rather than the snail's ability to form memory to reduce aerial respiration. We have also previously shown that the KCl stressor, which is a noxious stimulus, does not alter total breathing time in control preparations (Martens et al., 2007a; Martens et al., 2007b) whereas CE exposure does increase total breathing time in intact snails (Orr et al., 2007). However, as breathing rate is not altered in KCl-exposed snails, we conclude that the lack of memory enhancement in the absence of osphradial input to both the CE and KCl stressors is not due to a 'side-effect' of the stressors on the breathing rate.

Interestingly, the modulatory effects on LTM mediated by the afferent osphradial nerve input can be either enhancing (CE, KCl) or diminishing (low environmental Ca^{2+}) (Dalesman et al., 2011a; Dalesman et al., 2011b). This finding may allow us to better elucidate the mechanism(s) by which these various stressors alter memory formation at the single cell level. We know that the afferent input from the osphradium elicited by exposure to CE works *via* a serotoninergic pathway (Il-Han et al., 2010). Preliminary data (K.L., unpublished results) suggest that serotonin is also involved in the mediation of the enhanced LTM formation as a result of the KCl bath. We have not determined what transmitter(s) is used to cause the diminution of LTM formation by low environmental Ca^{2+}, which is also mediated by afferent input to the CNS from the osphradium (Knezevic et al., 2011; Dalesman et al., 2011a; Dalesman et al., 2011b). Both CE and KCl cause a significant decrease in RPeD1 excitability, which we hypothesize 'primes' RPeD1 to produce LTM. Low environmental Ca^{2+}, which also acts *via* the osphradium and osphradial nerve, prevents the decrease in RPeD1 activity usually seen with operant conditioning (Dalesman et al., 2011a; Dalesman et al., 2011b).

A 'primed' RPeD1 (i.e. significantly lower excitability) is hypothesized to be the reason why a single 30 min TS now is sufficient to result in LTM formation. We have arrived at this hypothesis based on both behavioral (Parvez et al., 2005; Parvez et al., 2006) and electrophysiological studies (Braun and Lukowiak, 2011). Parvez et al. showed that in snails receiving a single 30 min TS, only a memory persisting for 3 h was seen (Parvez et al., 2005; Parvez et al., 2006). However, if 24 h later the snail received even a single reinforcing stimulus, LTM was formed. The authors suggested that there was a 'residual memory trace' present which could be built upon to produce LTM when triggered by even a single 'poke' to the pneumostome seen (Parvez et al., 2005; Parvez et al., 2006). Braun and Lukowiak (Braun and Lukowiak, 2011) showed that a similar 30 min training procedure results in a 3 h memory but has an effect on RPeD1 activity, which is still apparent 24 h later. Despite no behavioral phenotype of memory, the excitability of RPeD1 was significantly decreased when compared with both the naïve and yoked control animals (although this was not as low as in preparations from snails exhibiting LTM). These same authors showed that with a training procedure that produces LTM there was a 'cellular fingerprint' of LTM in RPeD1 that correlated with behavioral LTM, i.e. RPeD1 was significantly less excitable (i.e. lower input resistance, excitability and bursting behavior) than what was seen 24 h after the single 30 min TS. The decreased RPeD1 activity found 24 h after the single 30 min TS might be the electrophysiological correlate of the 'residual memory trace'. Our working hypothesis is that the two stressors we tested here act *via* the osphradial pathway to create a state that resembles the 'residual memory state' observed in the Braun and Lukowiak (Braun and Lukowiak, 2011). If the osphradial input is interrupted, the stressors do not alter RPeD1 activity and thus LTM is not seen with a single 30 min TS.

Whilst the enhancement of LTM formation seen here is dependent on input from the osphradium, sensing different stressors may not be. For example, in the cut osphradial nerve snails, while KCL does not enhance LTM formation it still elicits the whole body withdrawal response of the snail (V.K., personal observation). This indicates that: (1) snails still sense and respond to the noxious KCl stimulus; (2) a different sensory pathway is involved in the mediation of the whole snail withdrawal response (i.e. input *via* the osphradial nerve is not required); and (3) sensing a noxious stimulus does not necessarily result in LTM enhancement.

We have previously demonstrated that two memory-blocking stressors (low environmental Ca^{2+} and crowding) that result in the same behavioral phenotype are not sensed *via* the same sensory pathway (Dalesman et al., 2011b). Here, we have demonstrated that whilst a stressor may be sensed by an animal (i.e. whole body withdrawal in response to KCl), it can alter different behavioral and physiological traits dependent on the specific sensory pathway. Therefore, we have demonstrated that the sensory mechanism for a particular stressor cannot be predicted based on the phenotype it produces (Dalesman et al., 2011b), and if an animal can sense a stressor it will not necessarily always alter their behavior in a predictable way. How the animal responds (e.g. with or without enhanced memory) will depend on the sensory pathway *via* which the stressful stimulus is sensed. When the effects of a stressor are assessed they are generally looked at the level of the whole organism; however, our data indicate that this will not necessarily elucidate the mechanism(s) by which the stress is modulating the behavioral and physiological traits.

FUNDING

This study was funded by an Alberta Heritage Foundation for Medical Research (AHFMR) postdoctoral fellowship awarded to S.D. and a Natural Sciences and Engineering Research Council of Canada (NSERC) Summer studentship to V.K. M.B. was funded by a Canadian Institutes of Health Research (CIHR) grant to K.L. NSERC funds to K.L. also supported V.K.

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