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RESEARCH ARTICLE

What's hot: the enhancing effects of thermal stress on long-term memory formation in *Lymnaea stagnalis*

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SUMMARY

The pond snail, *Lymnaea stagnalis*, naturally inhabits slow flowing, shallow and stagnant environments in the northern temperate zone. Consequently, it will experience wide temperature fluctuations dependent on prevailing weather conditions. We hypothesize that periods of warming act as a thermal stressor to alter memory formation. Snails were exposed to an acute 1 h period of 30°C pond water and we determined how memory formation following operant conditioning of aerial respiration was affected. In the snails used here (Dutch strain), a single 0.5 h training session (TS) results in intermediate-term (3 h) but not long-term memory (LTM). Applying the thermal stressor during training caused memory enhancement (i.e. LTM lasting 24 h). However, the breathing rate also increased in warm water, which might explain the enhanced memory. Therefore, we applied the thermal stressor (1 h at 30°C) up to 4 h before or 1 h after training. This did not alter baseline breathing rate during the period when snails would experience training. However, the thermal stressor whether experienced prior to or following the single TS, resulted in an enhanced memory that persisted up to 48 h (i.e. LTM). We conclude that memory enhancement is due to the stress associated with the thermal stimulus.

Key words: *Lymnaea*, long-term memory, thermal stressor, memory enhancement.

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INTRODUCTION

Memory formation is vital in allowing an individual to adapt its behaviour to current or future conditions. However, because memory is dynamic, stress can have a substantial modulatory effect on memory formation and retention (Kim and Diamond, 2002; Shors, 2004; Lukowiak et al., 2010). Therefore, experiencing stress, defined as any significant state that requires a physiological, psychological or behavioural readjustment or modification necessary to maintain the well-being of the organism (Selye, 1973; McEwen and Sapolsky, 1995; Lukowiak et al., 2008; Lukowiak et al., 2010), can alter an organism's ability to respond to its environment. The idea that stress alters memory formation and/or its recall is not new, as it was acknowledged by Bacon in the 17th century (Bacon, 1620). However, often it appears that there are contradictory data concerning stress and memory; sometimes stress enhances memory formation while at other times stress inhibits it. These apparent contradictions may be due to the complexities of using mammalian models, where, for example, maturity, sex, oestrus cycle, environmental conditions, maternal experience and previous experience of stress can all interact to alter how stressors affect learning and memory (Yang et al., 2003; Shors, 2004; Andreano and Cahill, 2009; Cacioppo and Hawkley, 2009; Conrad, 2010; Maeng and Shors, 2012).

We have focused our efforts to determine the causal mechanisms of how stress alters memory formation using the pond snail, *Lymnaea stagnalis*. *Lymnaea* are used to elucidate the relationship between stress and memory, because we have an excellent understanding of the underlying neuronal circuit that both drives aerial respiratory behaviour and is necessary for memory formation (Syed et al., 1990; Syed et al., 1992; Scheibenstock et al., 2002). Since *Lymnaea* are bimodal breathers (cutaneous and aerial respiration) it is possible to use an operant conditioning procedure to cause a decrease in aerial respiratory behaviour without harming the snail (Lukowiak et al., 1996). Retention of this behaviour is considered memory and it can be short-term (STM: lasting minutes), intermediate-term (ITM: lasting 2–3 h) or long-term (LTM: lasting >18 h) (Lukowiak et al., 2000). Using this relatively simple behaviour we can tightly control many of the factors that may confound results obtained in mammalian systems. This means we can accurately assess how stress alters behavioural and neurophysiological responses to a training protocol.

Exposure to various stressors around the time of the training procedure has been shown to affect the duration that the memory persists (Lukowiak et al., 2010). For example, low calcium availability or crowding blocks LTM formation (De Caigny and Lukowiak, 2008; Dalesman et al., 2011b), whereas exposing snails to the scent of a sympatric predator enhances LTM formation (Orr and Lukowiak, 2008; Orr et al., 2009). However, when stressors are combined the resulting effects on memory formation are difficult to predict due to emergent properties of the neuronal networks mediating the stress (Dalesman and Lukowiak, 2011). As a species that lives in shallow, often stagnant environments in the northern temperate zone (Boycott, 1936), *Lymnaea* will be exposed to broad temperature fluctuations dependent on prevailing weather conditions. For example, within a day in spring or autumn, snails may experience temperatures close to freezing over night and increasing to 20°C during the day, whereas in the middle of summer shallow water bodies will frequently warm to around 30°C in mid-afternoon (Brown, 1979). We therefore hypothesized that temperature may act as an environmentally relevant stressor that will affect learning and memory formation in *Lymnaea*.

Our previous work focused on the effects of cooling on memory formation. For example, cooling snails (to 4°C) for an hour after

training has been shown to block the formation of both ITM and LTM, probably due to inhibiting new protein synthesis that is a necessary component in the formation of memory lasting more than a few minutes (Sangha et al., 2003b). However, cooling *Lymnaea* to 4°C for just 10 min then rapidly warming them up back to 23°C either immediately prior to or following training enhanced their ability to form LTM, possibly due to stress from the rapid temperature fluctuation (Martens et al., 2007). Here we focus on how acute exposure to warm water (1 h at 30°C), as might be experienced in mid-summer, affects the ability of *Lymnaea* to learn and form LTM. We hypothesized that exposing the snails to this warm water would increase their metabolic rate and possibly enhance the ability of the snails to form memory. In *Caenorhabditis elegans*, it was found that memory consolidation was disrupted by heat during training, but not before or after training (Beck and Rankin, 1995); hence the timing of a heat stress may be integral to its effects on memory formation. Therefore we exposed *Lymnaea* to heat during, before or following training, to assess whether memory enhancement is due to a side-effect of altered breathing rate in warm conditions, or a product of experiencing acute heat exposure irrespective of baseline breathing behaviour.

MATERIALS AND METHODS

Animals

Lymnaea stagnalis, originally obtained from Vrije Universiteit, Amsterdam that originated from a strain of snails collected from canals in polders near Utrecht, The Netherlands in the 1950s, were raised in the Biological Sciences snail facility at the University of Calgary. Adult snails (with a shell length of 2.5–3.0 cm) were maintained in eumoxic (i.e. normal O_2 levels; P_{O_2}>9975 Pa) aquaria in artificial pond water (distilled water with 0.26 g l^{-1} Instant Ocean, Spectrum Brands, Madison, WI, USA). Additional calcium sulphate dehydrate was added to create what we refer to as a standard calcium level of 80 mg l^{-1} [Ca^{2+}] (Dalesman and Lukowiak, 2010; Dalesman et al., 2011b; Knezevic et al., 2011). Snails were maintained at room temperature (~20°C) and fed Romaine lettuce *ad libitum*.

Locomotory activity (crawling speed)

Individually marked *Lymnaea* were taken from their home aquaria and placed in a large Petri dish (14 cm diameter by 2 cm depth) in 200 ml of oxygenated artificial pond water (either at room temperature or 30°C) at a depth of 15 mm in the dish, sufficient to fully submerge the snail. Commencement of measurements of their crawling speed was initiated when the head and tentacles were fully extended. Once this occurred the distance moved was measured over a period of 15 min; a 2×2 cm grid marked on the base of the Petri dish allowed the distance crawled to be estimated by counting the number of squares each snail crossed during the 15 min observation period. The mean travelling speed over 15 min was then calculated in mm s^{-1} (Dalesman and Lukowiak, 2010). Snails were observed at room temperature as well as 4 h after a 1 h period at 30°C.

Breathing observation

Lymnaea are bi-modal breathers, able to directly absorb oxygen from the water via cutaneous respiration and respire aerially using a rudimentary lung opened to the atmosphere via the pneumostome. Pneumostome opening is an easily observable and recordable behaviour. In eumoxic conditions, the primary form of respiration is cutaneous; however, this becomes secondary to aerial respiration if the oxygen levels in the water are diminished (i.e. hypoxia). Pond water is made hypoxic (P_{O_2}<931 Pa) by bubbling N_2 vigorously through it for 20 min prior to breathing observation sessions. Duration of time spent aerially respiring is significantly increased by making the water hypoxic (Lukowiak et al., 1996).

Lymnaea breathing behaviour was recorded in freely behaving animals in hypoxic conditions for 30 min following a 10 min acclimation period. Stressors that alter LTM formation have also been found to alter breathing behaviour (Orr et al., 2007; Dalesman and Lukowiak, 2010). Alternatively, breathing behaviour may also be elevated by heat exposure, which has been found in other freshwater pulmonates (Calow, 1975). Therefore we measured aerial breathing behaviour under control conditions (normal laboratory temperature, 20°C), during exposure to warm water (30°C) and also in control conditions immediately following and 4 h following 1 h exposure to warm water. These data supplied a baseline breathing profile and allowed us to later determine if breathing behaviour was modified by the thermal stress.

Heating procedure

A tank of pond water heated to and maintained at 30°C served as a water bath to a 1 litre beaker filled with 500 ml of 30°C pond water. Up to 12 snails were kept in the beaker for the entirety of the 1 h heating period. This is referred to as the thermal stress. The thermal stress was given to snails at various times before, after or during training.

Operant conditioning

Operant conditioning to reduce aerial respiration in hypoxic conditions was carried out using a training procedure consisting of a single 0.5 h training session (Sangha et al., 2003a; Parvez et al., 2005; Orr and Lukowiak, 2008). N_2 was bubbled vigorously through 500 ml of artificial pond water in a 1 litre beaker for 20 min to make the water hypoxic. After 20 min bubbling was turned down to a low level to maintain hypoxia while not disturbing the snails. Snails were placed in the beaker and allowed to acclimate for 10 min prior to training. The training session (TS) was then carried out for 30 min, during which time the snail was gently prodded on the pneumostome using a wooden stick each time it attempted to open the pneumostome to perform aerial respiration. This ‘poke’ resulted in the closing of the pneumostome but not a full body withdrawal response. The snail was then returned to its home aquarium in eumoxic pond water. Snails were then tested for LTM formation 24, 48 or 72 h after TS. The 0.5 h memory test was carried out in exactly the same way as the training session. The snail was considered to have memory if the number of attempted pneumostome openings in the memory test (MT) was significantly lower than the number of attempted openings in the TS (Lukowiak et al., 1996; Lukowiak et al., 1998; Lukowiak et al., 2000). Yoked controls were carried out in the same hypoxic conditions and with the same acclimation period. However, the application of tactile stimuli to the pneumostome area was contingent not on the snails in the first group aerially respiring, but on the pneumostome opening of the snail to which the focal animal was ‘yoked’.

Statistics

A paired *t*-test was used to assess whether individual crawling rate differed dependent on temperature exposure (room temperature *versus* exposure to 1 h at 30°C 4 h prior to recording), and also whether breathing behaviour differed between animals maintained at room temperature or exposed to 30°C either during or prior to measuring breathing rate. Paired *t*-tests were also conducted to compare the number of attempted pneumostome openings during the training session (TS) and the memory test (MT) in the case of

operant conditioning training. Results were considered significant (i.e. LTM is present) if snails significantly reduced the number of attempted pneumostome openings between MT and TS. All statistics were calculated using InStat (GraphPad InStat version 3.0a for Macintosh, GraphPad Software, San Diego, CA, USA).

RESULTS

Aerial respiratory activity

A consequence of increased temperature experienced by the snails should be an increase in metabolic activity, which might result in an increase in respiration. We therefore tested aerial respiratory activity in hypoxia and measured total breathing time, number of breaths and the average length of each breath in a naïve cohort of snails (N=12; Fig. 1A–C). Snails were tested in hypoxic conditions at room temperature and the same animals were tested 24 h later at 30°C. Total breathing time was significantly increased (paired t-test, t=4.666, P<0.01) in the 30°C hypoxic condition compared with room temperature (Fig. 1A).

The average length of each breath (i.e. the time the pneumostome was opened) significantly decreased (paired t-test, t=5.616, P<0.01) in the 30°C condition (Fig. 1B); i.e. the average length of time of each breath decreased from ~50 s to 25 s. Since the total breathing time was significantly greater in the 30°C condition this meant that the number of pneumostome openings increased from ~10 at room temperature to 35 in the 30°C condition (Fig. 1C). Snails maintained in the 30°C condition have a significantly increased aerial respiratory profile compared with room temperature conditions.

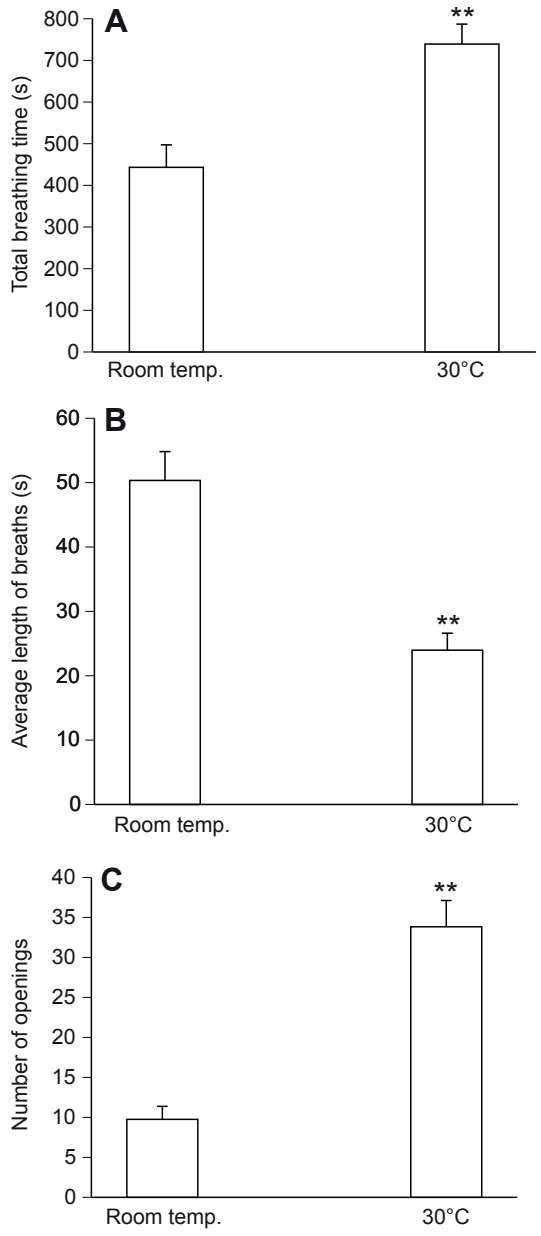

Fig. 1. The effect of temperature on aerial respiratory behaviours. (A) Snails in hypoxic warm pond water have a significantly increased total breathing time compared with their rates in hypoxic room temperature pond water. (B) The average length of each pneumostome opening was significantly shorter in hypoxic warm pond water compared with hypoxic room temperature pond water. (C) Snails had a significantly greater number of pneumostome openings in warm hypoxic pond water compared with the number of openings observed in room temperature hypoxic pond water; that is, snails 'pant'. Data are means ± s.e.m. (**P<0.01).

A single 0.5 h training session does not result in LTM

We next wished to determine if training snails in the 30°C pond water would alter their ability to form long-term memory (LTM). Naïve snails (N=20; Fig. 2A) were given a 30 min TS and were then tested for memory 24 h later (MT). The number of attempted pneumostome openings in the TS and MT were compared and did not differ significantly (paired t-test, t=0.43, P=0.671). These findings are consistent with previous experiments (Sangha et al., 2002; Parvez et al., 2005) showing that the laboratory-reared snails used here do not possess enhanced memory-forming capabilities, as opposed to some

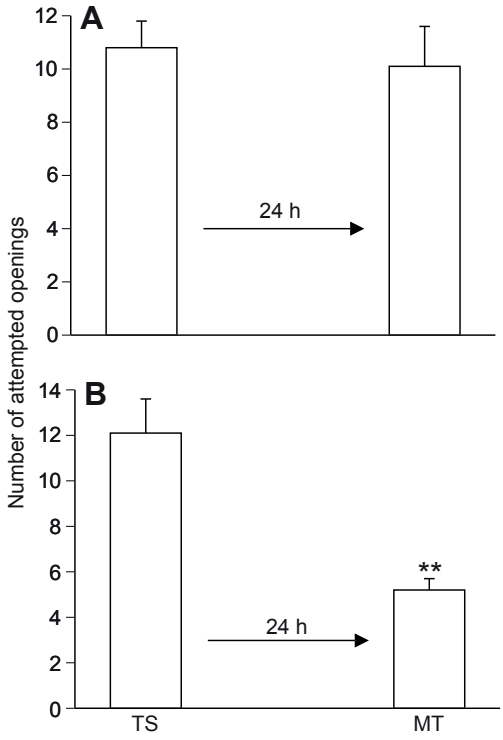

Fig. 2. The effects of a 0.5 h training session (TS) and memory test (MT) in different conditions. (A) A single 0.5 h TS at room temperature does not result in LTM formation in laboratory-reared snails. A naïve cohort of snails (N=20) that received a single 0.5 h TS did not exhibit memory when tested 24 h later (MT). (B) Snails that received a 0.5 h TS in 30°C pond water exhibited LTM when tested in 30°C pond water 24 h later (N=10). Data are means ± s.e.m. (**P<0.01).

populations of wild snails that show LTM following a single 0.5 h TS (Braun et al., 2012; Dalesman and Lukowiak, 2012).

A single 0.5 h training session in 30°C water results in LTM when tested in 30°C

Naïve snails (N=10, Fig. 2B) were given 0.5 h TS in 30°C pond water and then tested for memory 24 h later, also in 30°C pond water. The number of attempted pneumostome openings in the MT was significantly lower than the TS (paired t-test, t=3.88, P=0.0037). However, since breathing behaviour is significantly increased in warm water (Fig. 1), the enhancing effect seen here on memory formation in warm water may have been due to the tactile stimuli having a greater relevancy due to the animals' greater need to perform aerial respiration.

To determine if the thermal stressor causes enhancement of LTM formation we employed a different training and exposure to the thermal stressor procedure. In these experiments the thermal stressor (1 h in 30°C pond water) was presented to the snails either before or after the training session, which took place in room temperature pond water.

One hour at 30°C prior to training causes an enhancement of LTM

We first subjected snails to the thermal stressor before they received operant conditioning training. We found that this resulted in enhanced LTM formation (Fig. 3). We tested if LTM was present 24, 48 and 72 h later. As can be seen (Fig. 3A,B) LTM was present 24 h (paired t-test, t=3.22, P=.0005, N=20) and 48 h (paired t-test, t=2.2, P=0.043, N=9) later. Memory was not present 72 h after the TS (paired t-test, t=0.05, P=0.959, N=14) (Fig. 3C). Thus the presentation of the thermal stressor before training results in enhanced LTM formation.

One hour at 30°C after training causes an enhancement of LTM

We then examined whether the application of the thermal stressor after training would also result in enhancement of LTM formation. Forty naïve snails received a single 0.5 h TS in room temperature pond water and immediately following the TS they were placed in a 30°C eumoxic pond water aquarium for 1 h and tested for memory 24, 48 and 72 h later. Ten of these animals were tested for memory 24 h later and as can be seen in Fig. 4A, LTM was present (paired t-test, t=4.93, P<0.01). We then tested another group of snails (N=18) for memory 48 h later (paired t-test, t=2.7, P<0.01). As can be seen, LTM was present (Fig. 4B). The final 12 snails were tested 72 h later (Fig. 4C). LTM was also observed 72 h after training (paired t-test, t=3.08, P=0.011). Thus subjecting snails to the thermal stressor following training was more effective in enhancing LTM formation than presenting the stressor before training.

It could be argued that the thermal stressor applied immediately before or after training altered aerial respiratory behaviour such that the significant decrease in the number of attempted pneumostome openings was not actually memory but rather a recovery from the increased need to respire as a result of the immersion in the 30°C eumoxic pond water. To address this possibility we performed a series of experiments in which we examined a number of behaviours 4 h following the thermal stressor to determine if the snails behaved as though they were in an 'excited' state.

Heat stress and other behaviours 4 h later

Locomotory speed was determined in a naïve cohort of snails in room temperature pond water and in the same snails 24 h later, 4 h after a 1 h exposure to the thermal stressor. As can be seen (Fig. 5), locomotory speed 4 h after a 1 h thermal stress period does not differ significantly from the baseline locomotory speed determined in the same animals 24 h earlier (paired t-test, t=0.254, P=0.88, N=8). This suggests that 4 h after the thermal stress, the stimulatory effects of the stress have worn off.

To determine if the effects of warm water on breathing rates extended for a period of time beyond the period of thermal stress, a 1 h exposure to 30°C was applied directly before a breathing observation session (Fig. 6A) as well as 4 h before a breathing

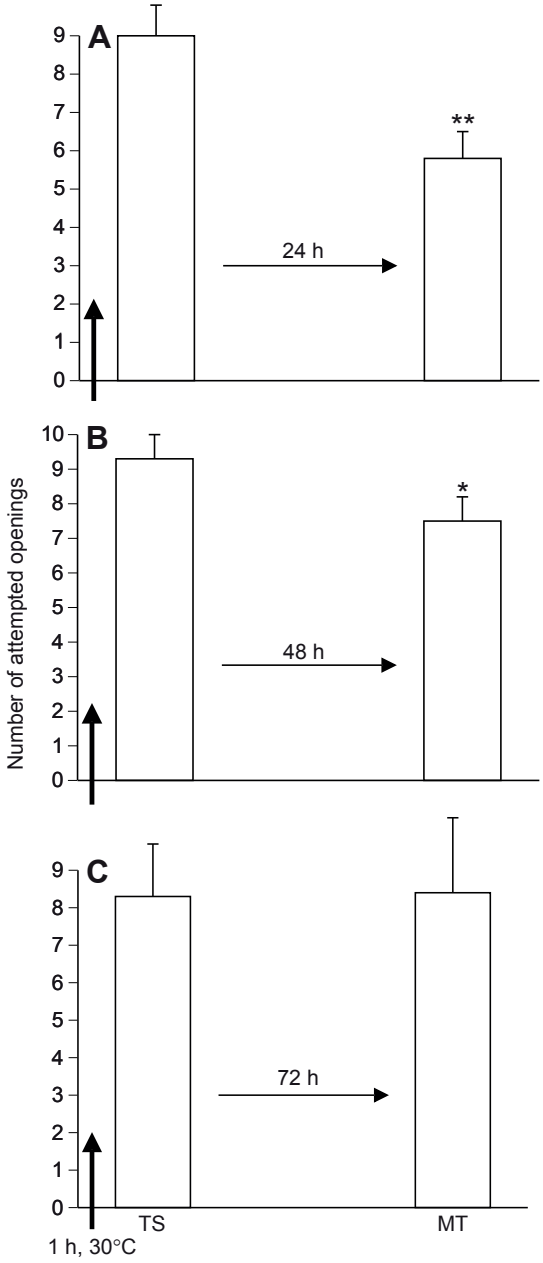

Fig. 3. The thermal stressor presented prior to training enhances LTM formation. (A) A naive cohort of snails (N=20) were exposed to the thermal stressor for 1 h prior to receiving the single 0.5 h TS. Memory was tested 24 h later and, as can be seen, LTM is present. (B) As in A (N=17) except memory was tested 48 h later. LTM was present. (C) As in A (N=9) but 72 h after TS, LTM was not present. Data are means ± s.e.m. (*P<0.05; **P<0.01).

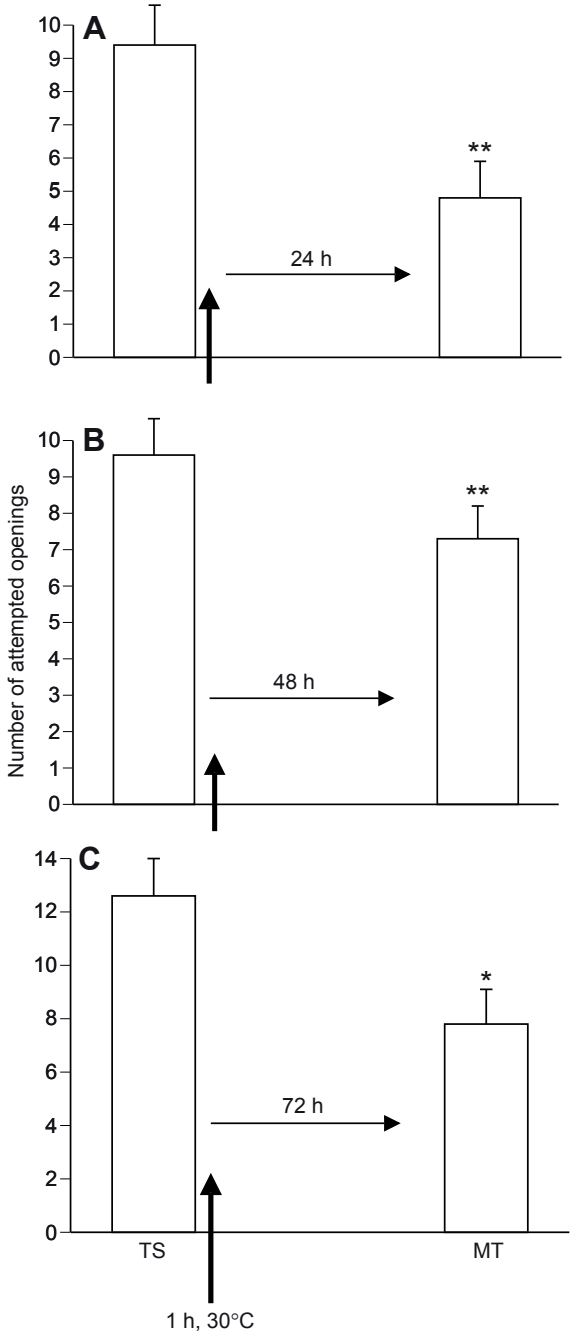

Fig. 4. The thermal stressor enhances LTM formation when applied to snails after a single 0.5 h training session. (A) A naïve cohort of snails (N=10) received a single 0.5 h TS in room temperature pond water. Immediately after training they were placed into 30°C pond water for 1 h. Following this they were returned to their holding aquarium at room temperature. When tested for memory 24 h later they exhibited LTM. (B) As in A (N=19) but memory was tested 48 h after TS. As can be seen, LTM was observed. (C) As in A (N=12) except memory was tested 72 h after TS. Memory was also present. Data are means ± s.e.m. (*P<0.05; **P<0.01).

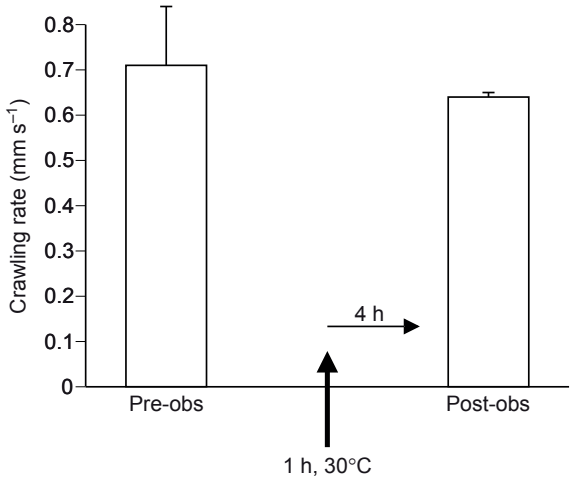

Fig. 5. Crawling rates are not enhanced following the presentation of the thermal stressor. Crawling rates were measured before the presentation of the thermal stressor (pre-observation) and 4 h after the thermal stressor (post-observation). There was no statistical difference in the crawling rates. Data are means ± s.e.m.

observation session (Fig. 6B). First, the total breathing time in a 30 min observation session in a naïve cohort of snails (N=9) was determined in hypoxic pond water at room temperature. Twenty-four hours later the snails were exposed to 30°C eumoxic pond water for a 1 h period. Directly following this exposure snails were moved to room temperature hypoxic pond water for a 10 min acclimation period and then the 30 min breathing observation session followed. There was no significant difference in the breathing time in room temperature water after the thermal stressor (paired t-test, t=2.714, P=0.664). In a second control experiment baseline breathing rates (i.e. pre-observation) were calculated in a group of 13 snails in hypoxic conditions. Twenty-four hours later these animals were given a 1 h period of thermal stress and then returned to their home environment at room temperature for 4 h. At the end of this 4 h period, a 30 min breathing observations session (i.e. post-observation) was carried out (Fig. 6B) There was no significant difference in the breathing time before the thermal stress and 4 h after the stress (paired t-test, t=0.438, P=0.665).

Thus both locomotory and aerial respiratory behaviours returned to pre-stressor levels 4 h after the thermal stress was presented to the snails. The question now became: would a similar procedure (i.e. thermal stress then a 4 h wait) still result in the enhancement of LTM formation?

One hour at 30°C 4 h prior to training causes an enhancement of LTM

To determine if the memory-enhancing effects of the thermal stress persisted after a period of rest, 14 snails were given a 1 h exposure to 30°C and then returned to their home environment at room temperature for 4 h. At the end of this 4 h period, the animals were given a standard 10 min acclimation period and 30 min training session. They were tested for and demonstrated LTM 24 h later (paired t-test, t=3.92, P=0.001; Fig. 7)

Yoked control snails do not exhibit LTM even though they receive the thermal stressor

A final control experiment was performed. In this experiment we used yoked control snails to demonstrate that only with the presentation of the heat stressor and contingent stimulation of the pneumostome as the snails attempts to open it would LTM be observed. Thus a naïve cohort (N=22; Fig. 8) of snails was randomly divided into an operant conditioning group (N=11) and a yoked control group (N=11). They received their respective ‘training’ in

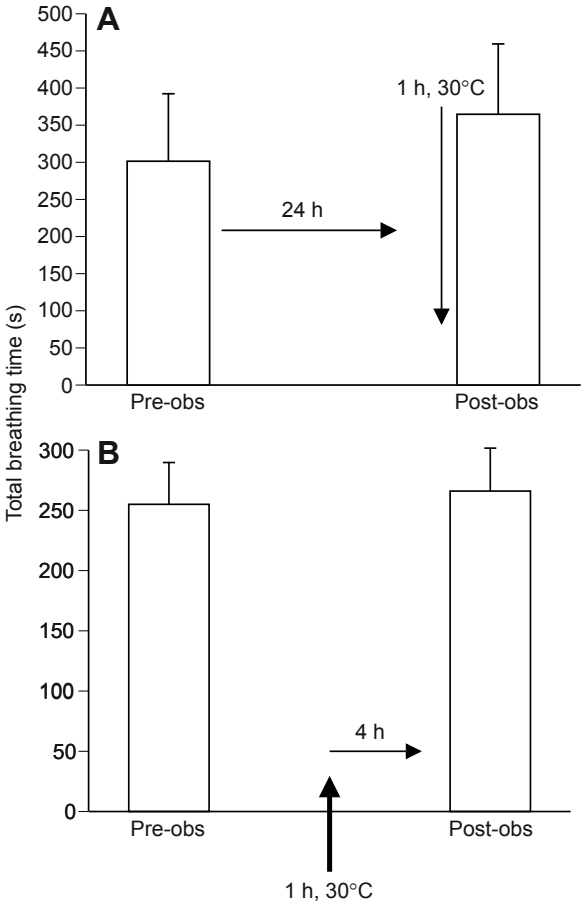

Fig. 6. Aerial respiratory activity after a 1 h thermal stressor. (A) The total breathing time observed in hypoxic room temperature pond water was not significantly altered by exposing snails to a 1 h thermal stress directly before observation. (B) In a similar manner the total breathing time was not altered when tested 4 h after the 1 h thermal stressor. Data are means ± s.e.m.

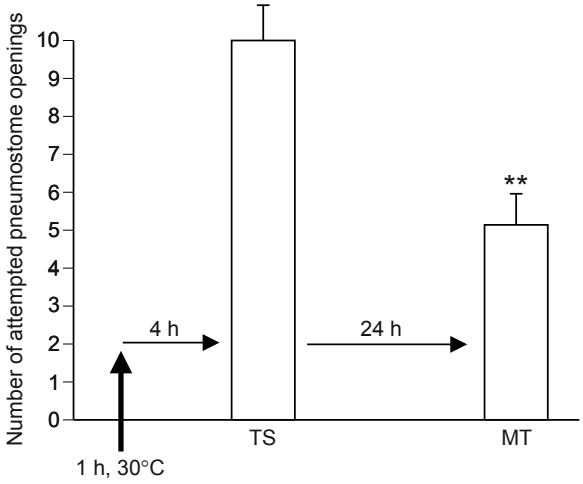

Fig. 7. The thermal stressor presented prior to training enhances LTM formation when snails are trained 4 h later. Naïve snails (N=14) were exposed to the thermal stressor for 1 h. Four hours later they received operant conditioning training. Memory was observed 24 h later. Data are means ± s.e.m. (**P<0.01).

room temperature pond water, then received the 30°C treatment for 1 h, and were tested for LTM 24 h later (Fig. 8). The yoked control snails did not exhibit LTM even though they received both the thermal stressor and the tactile stimuli, albeit in a non-contingent pattern (t-test, t=0.26, P=0.8).

DISCUSSION

Exposing *Lymnaea* to a brief period of heat (1 h at 30°C), either before, during or immediately after operant conditioning enhances LTM formation. However, yoked control snails exposed to the thermal stressor and receiving non-contingent tactile stimuli did not exhibit a change in the number of attempted pneumostome openings when we tested for memory. These data are all consistent with the hypothesis that the memory enhancement seen is due to the heat acting as a stressor on snails, which alters their ability to form memory following associative learning. A stressor is considered as any significant stimulus that requires a physiological or behavioural modification necessary to maintain the well-being of the snail (Selye, 1973; Lukowiak et al., 2008; Lukowiak et al., 2010).

Our previous work demonstrated that LTM in this species is modulated by environmentally relevant stressors (Lukowiak et al., 2010). Reviewing the literature on the response of *Lymnaea* to exposure to a 30°C thermal stimulus supports our hypothesis that the 1 h exposure to 30°C is perceived by snails as a significant stressor. In the laboratory, the maximum growth rate of *Lymnaea* is achieved between 11 and 28°C (Vaughn, 1953) and the lowest mortality occurs between 15.7 and 20.1°C (McDonald, 1969). Prolonged exposure to temperatures of 30°C or above is lethal (McDonald, 1973). In addition to the terminal effects of extended periods at 30°C, sub-lethal consequences have also been found. For example, Seppälä and Jokela (Seppälä and Jokela, 2011) showed that snails exposed to 30°C experience decreased resistance to pathogens, i.e. exposure to high ambient temperature appears to reduce snail immune function. In addition, previous work has shown that as the temperature during development increases, *Lymnaea stagnalis* reduces its innate response to predation (Dalesman and Rundle, 2010). Therefore, if freshwater environments experience greater temperature fluctuations and warmer summer temperatures in response to climate change, for example, this species may be less able to respond to predators. However, *Lymnaea* are also able to demonstrate learning about current predation risk, which may compensate fully for a low innate response (Dalesman et al., 2006; Dalesman et al., 2009). Therefore, whilst temperature elevation may reduce innate anti-predator behaviours, an enhanced ability to learn about predation threat may counteract this affect. The response of *Lymnaea* to acute elevated temperature in altering LTM formation is therefore consistent with our previous work, where acute exposure to stressors that can directly affect *Lymnaea* survival, growth and reproductive output also affect memory formation (Orr and Lukowiak, 2008; Lukowiak et al., 2010; Dalesman et al., 2011b).

Further evidence that temperature may act as a memory-enhancing stressor comes from work subjecting snails to a temperature alternation procedure (10 min of 4°C pond water followed immediately by a 10 min exposure to 23°C pond water) both before and after the single 0.5 h training session, which resulted in enhanced LTM formation (Martens et al., 2007). However, prolonged (60 min) exposure to 4°C directly after training blocked LTM formation (Sangha et al., 2003b; Sugai et al., 2007). This stress during the memory consolidation period was sufficient to block LTM while our 30°C stress given during consolidation showed memory-enhancing effects at least 24 h beyond what is seen when the thermal stress is given before training.

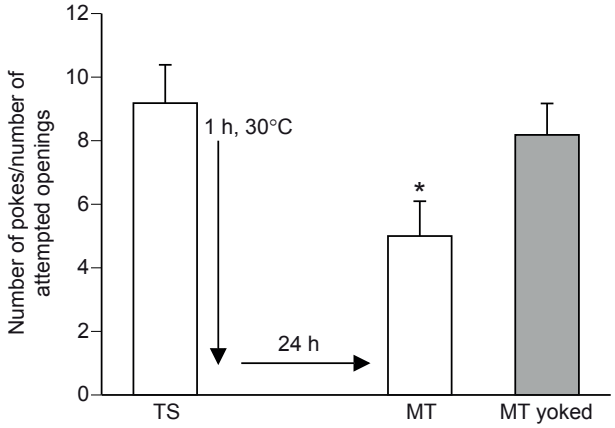

Fig. 8. Yoked control snails and thermal stress. Yoked control and operantly conditioned snails (N=12 in each cohort) received the single 0.5 h training session. Memory was tested 24 h later. Operantly conditioned snails exhibit LTM (MT) whilst yoked control snails (MT yoked) do not exhibit memory. Data are means ± s.e.m. (*P<0.05).

It is well known that *Lymnaea* living in their natural environment deal with a wide variation of temperature over both daily and seasonal time courses (Brown, 1979; Sidorov, 2003; Sidorov, 2005). Temperature can vary widely from 0 to 1°C (*Lymnaea stagnalis* in Southern Alberta have been observed performing aerial respiration in ponds with ice surrounding them) to 35°C (on hot summer days). Over the course of a single day temperature fluctuations of 4–28°C have been observed in shallow ponds in Southern Alberta (K. Lukowiak, personal observation). *Lymnaea* possess mechanisms for surviving in these varying conditions and the central nervous system (CNS) must play an important role in these processes. Effects of temperature on *Lymnaea* neurons and their synaptic connections have been determined for some of the neurons that are involved with aerial respiration (Sidorov, 2005). Sidorov found that snails kept for 2 weeks or more at water temperatures of 24–26°C did not alter the pattern of synaptic transmission between the neurons visceral dorsal 4 (VD4) and right pedal dorsal 1 (RPeD1). We have not yet attempted to determine how a step change in temperature (i.e. ~20–30°C) alters the electrophysiological properties and synaptic interactions between the respiratory central pattern generator (CPG) neurons and the output of these interneurons to the motor neurons. Both aerial respiratory behaviour and locomotory activity are increased in 30°C pond water. However, the 1 h exposure to the thermal stressor does not have a long-lasting effect on aerial respiratory behaviour *per se*, as aerial respiratory activity returns to normal levels following a 10 min exposure to room temperature. Thus the changes in CPG activity in 30°C pond water that drive the basic aerial respiratory behaviour are short lived.

How a thermal stress alters neuronal activity on a long-term basis has previously been studied in the ventilatory motor pattern generator of the locust, *Locusta migratoria* (Ramirez et al., 1999; Newman et al., 2003; Robertson and Money, 2012). Their basic finding was that tolerance to raised temperatures was the result of neurons employing homeostatic mechanisms to balance increases in K^+ outward currents by increasing ionic clearance mechanisms. This could involve increases in the activity of the Na^+/K^+-ATPase. We do not know whether such changes occur in *Lymnaea* following the thermal shock. However, if such long-lasting changes were to result in a decrease in the excitability of RPeD1, such a result would be consistent with our findings that RPeD1 is primed in 'smart' snails. That is, it appears as though a decrease in the naïve state excitability of RPeD1 accounts for the enhanced ability of 'smart' snails to make LTM (Braun et al., 2012).

We hypothesize that the 30°C thermal stressor session acts directly on neurons responsible for LTM formation and not *via* a peripheral sensory pathway. Previously we have shown that a peripheral sensory pathway mediated by the osphradial nerve is responsible for both the memory-enhancing effect of crayfish effluent (CE) and KCl, and the memory-suppressive effect of low environmental Ca^{2+} and heavy metals on LTM formation (Il-Han et al., 2010; Dalesman et al., 2011a; Byzitter et al., 2012; Karnik et al., 2012). However, we have found that the thermal stressor continued to result in enhancement of LTM formation in snails whose osphradial nerve had been severed (M.L.T., unpublished observations). We have not yet made electrophysiological experiments on a neuron (RPeD1) known to be necessary for LTM formation (Scheibenstock et al., 2002) following the 1 h 30°C thermal stressor session. However, we hypothesize that the thermal stressor session will cause RPeD1 to be in a state 'primed' for LTM formation, as we have seen with a strain of *Lymnaea* that exhibit superior memory-forming capabilities (Braun et al., 2012); that is, the electrophysiological state of RPeD1 following the thermal stressor session will be such that LTM is more easily formed. These experiments will be undertaken soon.

LIST OF ABBREVIATIONS

ITM	intermediate-term memory
LTM	long-term memory
MT	memory test
STM	short-term memory
TS	training session

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