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Research report

Ecologically relevant stressors modify long-term memory formation in a model system

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ABSTRACT

Stress can alter adaptive behaviours, and as well either enhance or diminish learning, memory formation and/or memory recall. We focus attention on how environmentally relevant stressors (e.g. predator detection, crowding, and low concentrations of environmental Ca^{++}) alter memory formation in the pond snail, *Lymnaea stagnalis*. We specifically look at operant conditioning of aerial respiration and whether or not long-term memory forms following the acquisition of the learned event, not performing aerial respiration. We will also examine the strain differences in Lymnaea which allow or cause isolated populations to possess different heritable cognitive capabilities, as manifested by differing abilities to form long-term memory.

It is well known that stress modulates memory formation and/or its recall. This 'fact' has been noted in the scientific literature since the time of Bacon [1]. The best summary of this is the so-called Yerkes–Dodson 'law' ([2,3]; Fig. 1). Organisms should only expend the 'neuronal cost' (i.e. altered gene activity and new protein synthesis) necessary to form LTM to 'relevant' events. An important factor in determining whether an event will be encoded into memory is the level of stress around the time of the event. Stress is any significant condition that necessitates physiological, psychological or behavioural readjustment or modification that is necessary for the well-being of the organism [4,5]. Stressors can take the form of either physical (e.g. heat shock) or psychological (e.g. public speaking) challenges and have the ability to alter the processes of memory formation and recall [2,6]. Depending on the specific stressor and how the stress is perceived, memory formation or its recall may be enhanced or impaired (e.g. [7–10]). Stress has the ability to modulate memory as memory is a dynamic brain process [11,12]. Stress may also play a role in false memory formation and PTSD [13–15]. The various effects of stress on memory have been studied in a number of different model organisms, with sometimes contradictory results [3]. That is, in some instances memory is enhanced while in others its formation or its recall is blocked. Given the complexities of the vertebrate brain and animal behaviour, and the diverse ways stressors act on memory, disagreement in the literature is not surprising. We believe that we can overcome many of these obstacles by using a molluscan model system, the pond snail, *Lymnaea stagnalis*.

The vast majority of memory studies (see [16,17]) involving Lymnaea have utilized laboratory-bred specimens derived from snails originally collected (in the 1950s; >250 generations in the lab) from canals near Utrecht in The Netherlands. These are referred to as 'lab-bred' snails. However, we have begun to use freshly collected and F1s and F2s of Lymnaea obtained from ponds in Alberta and in Southwest England (the Somerset Levels) that give us insight into how different populations have differing capacities to form memory and deal with stress. In this review we will: (1) focus our attention on how ecologically relevant stressors alter long-term memory (LTM) formation; (2) outline our new research direction attempting to understand how instinct is embedded in the nervous system and the neuronal basis of strain differences in LTM-forming capabilities.

We will concentrate on one specific behaviour in Lymnaea; aerial respiration. Lymnaea satisfies its respiratory needs either cutaneously, through the skin, or aerially through the pneumostome, the respiratory orifice [18]. Aerial respiration, which involves opening the pneumostome, at the water's surface to allow atmospheric gas exchange, is driven by a three-interneuron central pattern generator (CPG) of which sufficiency and necessity have been directly demonstrated [19,20]; Fig. 1). This behaviour can be modified by operant conditioning [21–23]. Operant conditioning of aerial respiratory behaviour in Lymnaea is performed by placing snails into a beaker filled with hypoxic pond water (PW). The PW

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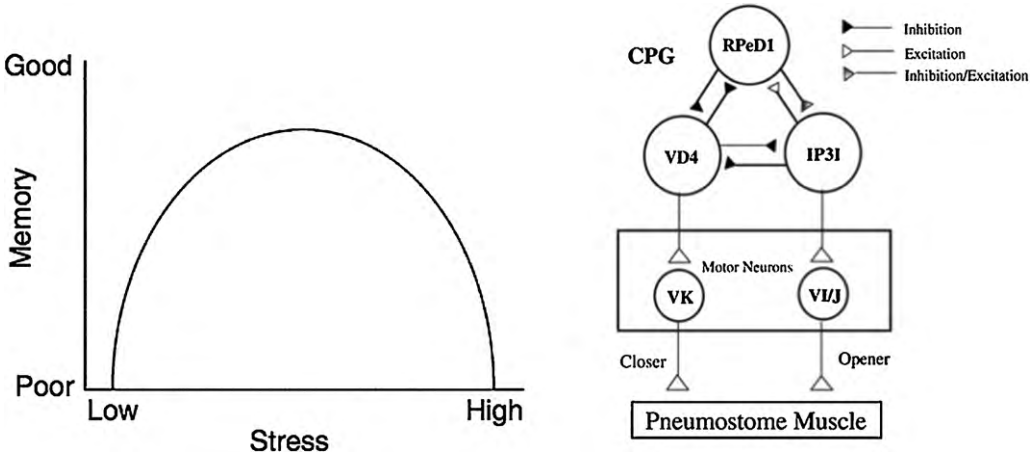

Fig. 1. The 'Yerkes–Dodson law' (left) demonstrates a relationship between stress and memory. Memory formation gets better with increasing stress, but only to a certain point: when levels of stress become too high, the ability to form memory decreases. (Right) Diagram of the three-neuron respiratory CPG and their follower cells. Depolarization of RPeD1 excites IP3 (inhibition followed by excitation) and inhibits VD4 (inhibition). Once activated, IP3 excites RPeD1 and a group of motoneurons (VI/J cells) involved in pneumostome opening. IP3 also inhibits VD4, the interneuron involved in pneumostome closing. The combined inhibitory input from both IP3 and RPeD1 causes VD4 to fire a burst of action potentials. This bust of activity in VD4 inhibits both RPeD1 and IP3 and excites a group of motoneurons (VK cells) involved in pneumostome closing. This begins the cycle of alternate bursting activity in VD4 and IP3 that underlies the generation of the respiratory rhythm.

is made hypoxic by bubbling N_2 gas through it for 20 min. Hypoxia is used to increase the snails' drive to perform aerial respiration. In hypoxic conditions, snails crawl to the air–water interface to open their pneumostome and perform gas exchange with the external environment. When they open their pneumostome we apply a negative reinforcement in the form of a gentle tactile stimulus. This reinforcing stimulus is of sufficient strength to cause pneumostome closure but does not elicit the whole-animal withdrawal response. With repeated application of the negative reinforcement, snails reduce the number of attempted pneumostome openings when placed in the hypoxic context. We defined learning and memory operationally. Learning is present if the number of attempted pneumostome openings in the last training session is significantly less than the number of attempted pneumostome openings in the first training session. In order for memory to be present when 'savings' is tested (memory test, MT), two criteria must be met: (1) the number of attempted pneumostome openings in the MT is not significantly greater than that of the last training session and (2) the number of attempted pneumostome openings in the MT is significantly less than that of the first training session. Depending on the training procedure used, intermediate-term memory (ITM; persists for up to 3 h and depends on de novo protein synthesis) or LTM (persists for more than 6 h and depends on both altered gene activity and de novo protein synthesis) can be formed [24–26]. In fact, molecular changes in one of the three CPG neurons, RPeD1, have been shown to be absolutely necessary for LTM formation, extinction, memory reconsolidation and forgetting [27–32]. We thus have the opportunity to determine how stress in Lymnaea alters LTM formation at the single neuron level.

One ecologically relevant stressor that will be discussed is exposure to the 'smell' of a crayfish predator. To obtain this 'smell', which we call crayfish effluent (CE), crayfish are maintained in aquaria and we used the water taken from the aquaria to train the snails in. Thus, snails did not come into direct contact with the predator. Predator detection in lab-bred Lymnaea elicits a suite of so-called vigilance behaviours indicating that the snail becomes stressed when it detects a predator [33]. A second stressor is overcrowding. Overcrowding alters genomic and behavioural activity in both vertebrates and invertebrates [34,35]. Crowding in Lymnaea significantly alters genomic activity, resulting in decreased growth rate, altered embryonic development and reproduction [36–39]. In crowded conditions, snails are maintained at a density of 20 snails per 100 ml of PW (normal density 2/100 ml). The third stressor used was low $[Ca^{++}]$ pond water. The pond water (PW) was made from de-ionized water to which we added calcium sulphate to make low calcium 20 mg/l $[Ca^{++}]$ or normal calcium 80 mg/l $[Ca^{++}]$ PW. Lymnaea are highly dependent on external calcium availability for survival, demonstrating reduced growth rate, survival and reproductive output in low calcium environments.

We used a number of different strains of Lymnaea in addition to lab-bred snails. We define a strain as that population of Lymnaea coming from the same body of water (e.g. pond or drainage ditch). The first strain was the 'Dutch' snails; three strains were Alberta snails and two strains came from the Somerset Levels. Our Dutch snails have been reared under lab conditions since the 1950s. All snails are *Lymnaea stagnalis* the only difference being what geographic location they were obtained from. Similar data were obtained from each strain regardless of whether they were freshly collected or whether they were F1s or F2s reared in the lab from eggs.

1. Predator detection

In the summer of 2006 we collected a strain of *Lymnaea stagnalis* from an interconnected series of ponds in Southern Alberta. What amazed us was that these snails had enhanced memory-forming capabilities compared to our Lab-reared snails ([40–43]; Fig. 2). Our initial hypothesis was that the Belly snails were reared in an enriched environment compared with the lab-reared snails. The obvious experiment then was to enrich the environment in the lab. We choose to do this by placing a predator (a crayfish a fortuitous choice) in the aquarium along with the snails. When we did this we found that the snails trained in crayfish effluent (CE), had enhanced memory-forming capabilities that while not quite as good as the Belly snails were much better than anything we ever saw from our lab-reared snails. Thus, it appeared that our hypothesis was proven. Except for one finding eggs collected from Belly snails and reared in the lab continued to have superior memory-forming capabilities compared to lab-reared snails or lab-reared snails trained in CE.

As can be seen in Fig. 3 when lab-reared snails detect the presence of a predator (i.e. trained in CE) they have enhanced LTM [40]. This is also evident at the electrophysiological level in recordings made from RPeD1. How do we know that detection of a predator is a stressful situation for the snail? We found [33] that CE elicits a change (a heightened response) in the snails so-called 'vigilance' or 'risk assessment' behaviours. For example, snails in CE respond to

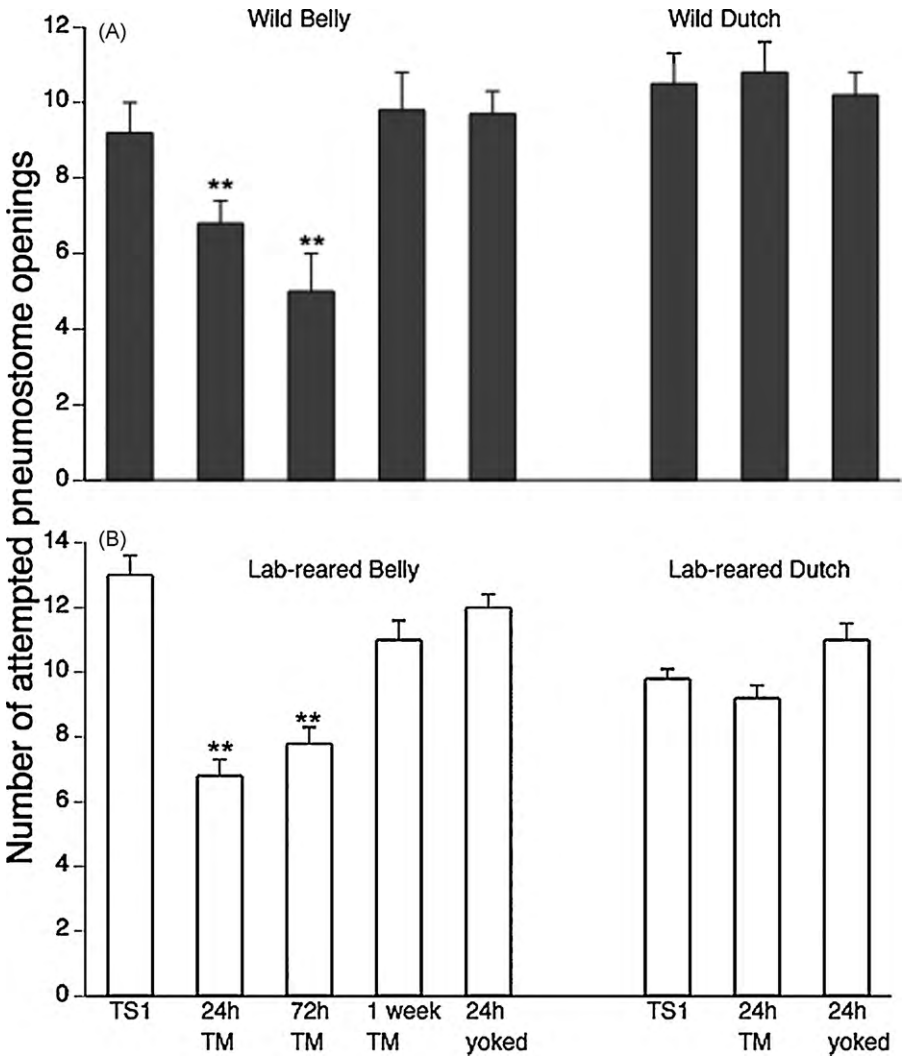

Fig. 2. Memory formation in Belly Dutch snails after a single 0.5 h training session (TS1). Operant conditioning of wild Belly snails results in an LTM that persists for 24 and 72 h. Yoked control snails do not demonstrate memory at these same time periods. Snails did not demonstrate memory after 1 week. Dutch lab-reared snails do not demonstrate LTM after a single 0.5 h training session. Wild Dutch snails do not demonstrate LTM when tested 24 h after operant conditioning. Yoked controls do not demonstrate altered behaviour after conditioning. Operant conditioning of Belly F1 snails results in an LTM that persists for 24 and 72 h. Results are shown as means + s.e.m. $^{**}P < 0.001$.

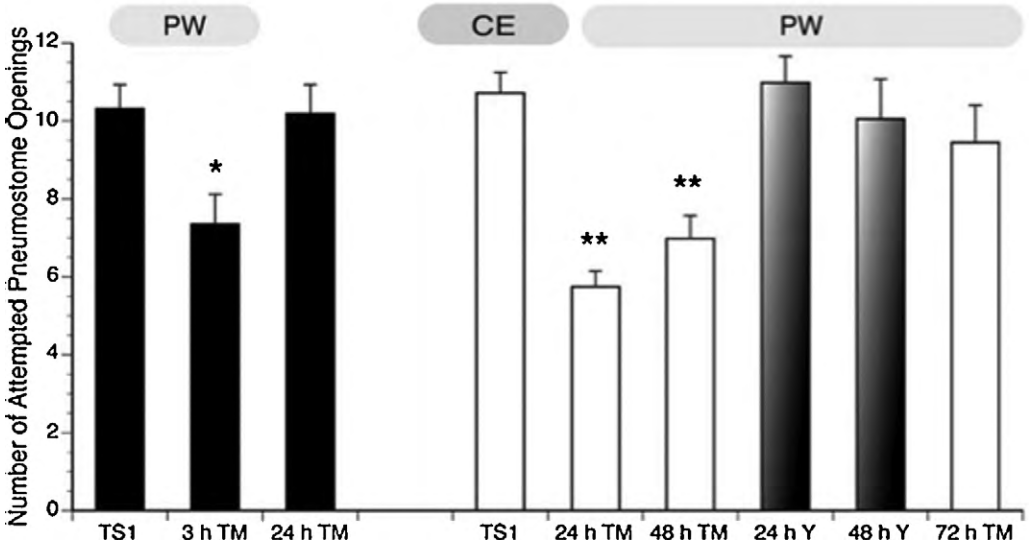

Fig. 3. Lab-reared Lymnaea after a single 0.5 h training session in either PW or CE. The single training session (TS1) in PW (black bars) results in a 3 h memory but does not result in LTM. However, the single training session (TS1) in CE results in LTM at 24 and 48 h, but not in 24 and 48 h yoked control groups or 72 h after training. $^{**}P < 0.001$.

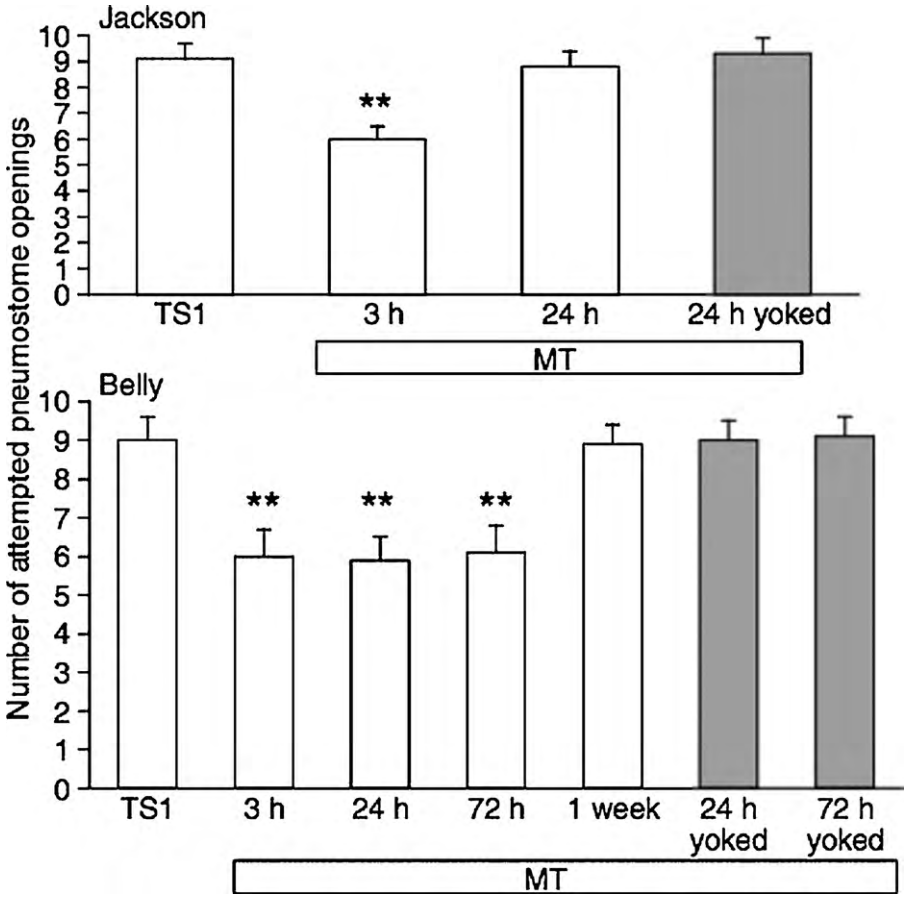

Fig. 4. Jackson and Belly snails. Jackson snails were given a single 0.5 h training session. Memory in one cohort was tested 3 h after TS1 and showed memory. A second cohort was tested 24 h after TS1 and did not exhibit memory, and neither did the yoked control. Bottom, Belly also received a single 0.5 h training session (TS1) and separate cohorts exhibited memory at 3, 24 and 72 h later. The 1-week cohort and the 24 and 72 yoked control cohorts did not exhibit LTM ($^{**}P < 0.01$).

a shadow stimulus with a much exaggerated withdrawal response than in PW. Hyper-arousal is considered a behavioural phenotype associated with stress. An alternative explanation was that a smell from any predator would elicit similar responses. This was not the case as tiger salamander effluent (SE) did not elicit vigilance behaviours in our lab-reared snails nor did it enhance LTM formation ([42]; see below). Tiger salamanders are not a sympatric predator of Dutch snails. Thus, we feel confident in concluding that CE elicits a stress in lab-reared Lymnaea.

We discovered a second pond closer to our lab (the Jackson pond) that also contained Lymnaea. These Lymnaea, however, did not possess the superior memory-forming capabilities that the Belly snails had. Jackson snails have the same memory-forming capabilities that our lab-reared snails had ([42,43] Fig. 4). We attempted to enhance their LTM-forming capabilities by training them in CE. CE, however, did not elicit vigilance behaviours nor did it enhance LTM! Crayfish, however, are not present in Alberta (i.e. not a sympatric predator). Thus, we attempted to see if a sympatric predator, Tiger salamander, altered LTM in Jackson snails. Pond water from the salamander aquarium (SE) enhanced LTM formation. Remember, SE did not enhance LTM in the Dutch snails and CE did not enhance LTM in Jackson snails, but in both strains the 'smell' (known as a karimore) from a sympatric predator enhances LTM formation (Fig. 5).

We next collected snails from the same area in The Netherlands where the founders of the colony were collected in the 1950. We tested these freshly collected 'wild' snails and found that their LTM-forming abilities were no different from our lab-reared snails (Fig. 2). These freshly collected snails also responded to CE with enhanced LTM but did not show enhanced LTM with SE. Thus, we concluded that detection of a sympatric predator alters the stress-state of the snail such that it allows for enhanced LTM; while the scent of a non-sympatric predator does not. Interestingly, in CE treated snails that exhibit enhanced LTM they lose their context-specific memory [44]. Stimulus generalization can be considered as another indication that CE increases stress levels.

2. Crowding

Crowding for as little as 1 h immediately before or after operant conditioning is sufficient to block LTM but not ITM formation. We also found that crowding for up to 23 h did not prevent snails from recalling an already formed LTM. As LTM requires both altered gene activity and new protein synthesis, whereas ITM requires only new protein synthesis, we hypothesize that crowding interferes with the necessary genomic activity to produce LTM in neurons, such as RPeD1, that are necessary for LTM formation. Whether chronic crowding (i.e. days to weeks) would have any different effect(s) on aerial respiration and/or memory formation remains to be determined.

Our study is the first we know of using crowding as a stressor to block LTM formation in a model system where it may be relatively easy to demonstrate how this stressor acts at the single cell level. For crowding to block LTM formation it must occur either immediately before or after the training procedure and it can be as short as 1 h. These data are consistent with the notion that memory modification is time dependent, i.e. it does not occur instantaneously [45]. We initially hypothesized that, as for some other stressors [46–48],

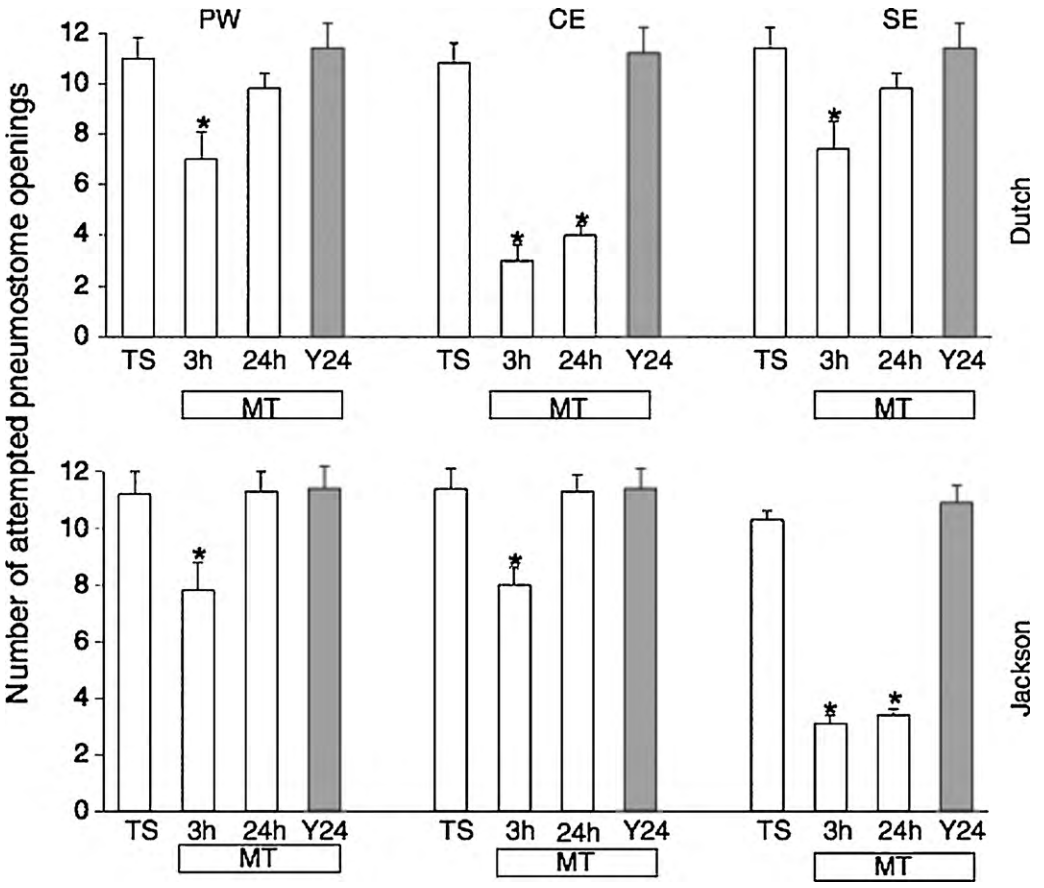

Fig. 5. Operant conditioning of Wild Dutch and Jackson snails. Each of these strains was trained in either pond water (PW), crayfish effluent (CE), or Tiger salamander effluent (SE). Top row: Dutch snails received a single 0.5 h training session in PW (left panel) and showed intermediate-term memory (ITM) but not LTM. Yoked controls also did not demonstrate LTM. Middle panel: Dutch snails that received a single 0.5 h training session in CE demonstrated both ITM and LTM. Yoked controls (Y24) in CE do not demonstrate LTM. Right panel: Dutch snails that received a single 0.5 h training session in SE were similar to those trained in PW, in that they demonstrate ITM but not LTM. Bottom row: Jackson snails received a single 0.5 h training session in PW demonstrated ITM but not LTM. Middle panel: Jackson snails that received a single 0.5 h training session in CE demonstrated ITM but not LTM. Right panel: Jackson snails that received a single 0.5 h training session in SE demonstrated both ITM and LTM Yoked controls in SE did not demonstrate LTM ($^{*}P < 0.01$).

crowding would impair memory recall. However, this was not the case. As LTM was still present in the memory recall experiments, we concluded that the ability of crowding to block memory formation was therefore not the result of a deficit in a general metabolic process that caused snails to perform aerial respiration to a greater degree and thus mask LTM. Earlier researchers [37] hypothesized that the reduced growth of snails in crowded conditions was due to a 'factor' in the water that the snails were maintained in. Voronezhskaya et al. [49] also found that 'chemicals' released into the water by snails in crowded conditions delayed embryonic development. Our initial assumption [50,51] was that water from the crowded snail aquarium would be sufficient to block LTM formation. However, our attempts to block LTM formation with pond water taken from a crowded aquarium (CPW) were unsuccessful. While it is possible that a water-borne factor might have a short half-life we do not believe that this is the case, unless of course the half-life is on the order of a minute or so. We believe this because we can upset memory formation with other stressful stimuli if applied within 5 min of the training event. It is possible that substances released by other live snails in their mucus may contain the substance(s) sensed by snails that causes LTM to be blocked.

3. Low levels of [Ca^{++}] in the environment

Calcium availability is considered to be the major limiting factor affecting the distribution of freshwater aquatic organisms including snails [52]. Snails rely on calcium for growth of their shell and are highly dependent on calcium for survival. In addition in low calcium (<20 mg/l) snails demonstrate thinning of the shell, potentially making them easier prey [52]. In high calcium environments (>80 mg/l) snails demonstrate induced shell thickening in the presence of predators, reducing predation mortality [53]. Widespread decline of calcium [54] has occurred in freshwater systems in North America, primarily due to leaching of calcium cations from soil following prolonged exposure to acid rain. Thus, reduced calcium in pond water is an environmental stressor.

Lymnaea are considered to be calciphiles in that they require at least 20 mg/l of environmental calcium as they are unable to satisfy their calcium requirements from their food [55]. So, do the calcium levels change in ponds and/or snail raising facilities where we get our snails from? *Lymnaea stagnalis* populations in drainage ditches on the Somerset Levels, UK, experience 8-fold changes in [Ca^{++}] from 23 to 185 mg/l (S. Dalesman, unpublished) over the course of a 3-month period. Macan [56] further demonstrated a 2–3-fold decline in environmental calcium in less than 1 week following periods of heavy rainfall. We used de-chlorinated Calgary city tap water until the summer of 2008. The calcium content varies significantly over the year (2-fold change). We found [55] that the speed of locomotion was significantly reduced in snails exposed to low environmental calcium (20 mg/l) compared to snails exposed to the high (80 mg/l) calcium environment. The slower speed observed in the low calcium is due to increased metabolic demands on Lymnaea, where a greater proportion of energy is required for calcium acquisition, reducing energy available for motility. We fur-

ther found that in low calcium there was a significant increase in cutaneous respiration (i.e. oxygen consumption was higher), consistent with our hypothesis that the increased metabolic demands of calcium acquisition at low [Ca^{++}] reduce the energy available for locomotion. Thus exposure to a low [Ca^{++}] environment alters basic physiological and behavioural traits, respiration and locomotion [55].

Does low environmental levels of calcium alter the ability to form LTM? Before answering that question it is important to note two important facts. First, low environmental [Ca^{++}] does not appear to alter haemolymph (i.e. blood) levels of [Ca^{++}]. Aquatic gastropods (e.g. Lymnaea) are able to maintain haemolymph calcium levels in a low calcium environment [57–59], even in the complete absence of environmental calcium for at least 10 days [60]. Second, Lymnaea appear to be able to detect low environmental levels of [Ca^{++}] as evidenced by their ability to orient themselves towards high [Ca^{++}] environments when given a choice [61]. Thus, any alteration in memory-forming capacity as a result of exposure to a low [Ca^{++}] environment would not be caused by a change in internal [Ca^{++}] levels effecting, for example, synaptic transmission; but rather would be due to the snail detecting that it is in a low [Ca^{++}] environment. On sensing the low [Ca^{++}] environment snails turn on mechanisms (e.g. calcium pumps) that begin to move Ca^{++} from the shell into the haemolymph in order to maintain Ca^{++} homeostasis. Presumably the turning on of pumps, etc. to maintain Ca^{++} homeostasis involves altered gene activity in neurons that control these processes (e.g. altered production of neurohormones). Preliminary data indicate that the sensing of the low calcium environment is sufficient to block LTM formation, but not its recall. Data show that even an exposure of only 1 h in low calcium before and during operant conditioning training is sufficient to block LTM. However, after LTM has formed placing the snails in the low [Ca^{++}] environment does not alter the ability of the snail to recall memory. These data suggest to us that the molecular changes induced in the snail by detection of low [Ca^{++}] are incompatible with the necessary gene-dependent processes underlying LTM formation. Recall of an already formed does not appear to be negatively impacted by the mechanisms necessary to maintain Ca^{++} homeostasis. In Drosophilia there is a cost of LTM formation, a shorter life span [62]. Since there is increased metabolic demand in the low calcium environment metabolically intensive processes such as LTM formation may be curtailed. Further study to test this hypothesis is underway. If correct we would then be interested to see if a low calcium environment will block the reconsolidation process.

4. Strain differences

In relating the story of how we stumbled on predator detection enhancing LTM formation it was mentioned that a strain of Lymnaea, the Belly snails, was found that had enhanced LTM-forming capabilities [42]. We have also investigated two other strains of Lymnaea in Alberta. The Jackson strain [42,43] has memory-forming capabilities similar to the freshly collected Dutch and lab-reared Dutch snails (i.e. not superior). Another stain is called TransCanada #1 (TC#1; a pond along side the Trans Canada Highway on the way to Banff) and it appears that the TC#1 snails are similar to the Belly snails regarding their superior memory-forming capabilities. The superior memory-forming capabilities of the Belly snails are heritable, in that F1 and F2 offsprings from the Belly snails reared under laboratory conditions continue to exhibit superior memory-forming abilities. We presume that this will also be the case for the TC#1 snails, but have not done those experiments. In the UK we have investigated 2 different strains (unpublished observations). Two of these strains (Chilton and South Drain) are from drainage ditches within 500 m of each other in the Somerset Levels of England. Remarkably, these 2 strains have differing LTM-forming capabilities. The Chilton strain, have enhanced LTM-forming capabilities similar to the Belly and TransCanada #1 snails; while the South Drain strain, have LTM-forming capabilities that are not different from the Lab-reared; freshly collected Dutch and Jackson snails.

5. Future directions

Where do we go from here? Two obvious paths are: (1) determine if the strains that possess enhanced LTM-forming capabilities are more or less resistant to stressors such as low environmental calcium or overcrowding; (2) cross breed the strains. For example, if we cross breed an Alberta 'smart' strain with a UK or Netherlands 'not so smart' strain we can ask a number of questions: (1) what do the offspring recognise as a predator (crayfish, tiger salamander, both, neither?); (2) will predator detection enhance LTM; (3) do all offspring possess superior LTM-forming capabilities? The other thrust will look at whether the superior LTM-forming strains are better able to cope with the various stressors. It may be that a reason these strains have better forming LTM capacities is that they are less prone to 'be stressed out. If so, we can begin to determine at the neuronal level why this is.

Thus, in this tribute to Brian Kolb, who pioneered the study of how the brain can respond to insults and recover from them, we have attempted to show that using the lowly pond snail one can ask and answer questions that are important for all of us.

References

[1] Bacon F. In: Anderson FH, editor. The new organon. New York: Bobbs-Merrill; 1620 [1620/1960].
[2] Yerkes RM, Dodson JD. The relation of strength of stimulus to rapidity of habit-formation. J Comp Neurol Psychol 1908;18:459–82.
[3] Shors TJ. Learning during stressful times. Learn Mem 2004;11:137–44.
[4] Selye H. The evolution of the stress concept. Am Sci 1973;61:692–9.
[5] McEwen B, Sapolsky R. Stress and cognitive function. Curr Opin Neurobiol 1996;5:205–16.
[6] Lukowiak K, Martens K, Rosenegger D, Browning K, de Caigny P, Orr M. The perception of stress alters adaptive behavious in *Lymnaea stagnalis*. J Exp Biol 2008;211:1747–56.
[7] de Quervain DJF, Roozendaal B, McGaugh JL. Stress and glucocorticoids impair retrieval of long-term spatial memory. Nature 1998;394:787–90.
[8] Bowman RE, Beck KD, Luine VN. Chronic stress effects on memory: sex differences in performance and monoaminergic activity. Horm Behav 2003;43:48–59.
[9] Cahill L, Gorski L, Le K. Enhanced human memory consolidation with post-learning stress: interaction with the degree of arousal at encoding. Learn Mem 2003;10:270–4.
[10] Joels M, Pu Z, Wiegert O, Oitzl MS, Krugers HJ. Learning under stress: how does it work? Trends Cogn Sci 2006;10:152–8.
[11] Hebb DO. The Organization of Behavior. New York: Wiley; 1949.
[12] Lewis DJ. Psychobiology of active and inactive memory. Psychol Bull 1979;86:1054–83.
[13] Kim J, Diamond D. The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 2002;3:453–63.
[14] Lukowiak K, Fras M, Smyth K, Wong C, Hittel K. Reconsolidation and memory infidelity in Lymnaea. Neurobiol Learn Mem 2007;87:547–60.
[15] Yehuda R, LeDoux J. Response variation following trauma: a translational neuroscience approach to understanding PTSD. Neuron 2007;56:19–32.
[16] Benjamin PR, Staras K, Kemenes G. A systems approach to the cellular analysis of associative learning in the pond snail Lymnaea. Learn Mem 2000;7:124–31.
[17] Parvez K, Rosenegger D, Martens K, Orr M, Lukowiak K. Learning at a snail's pace. Can J Neurol Sci 2006;33:347–56.
[18] Lukowiak K, Ringseis E, Spencer G, Wildering W, Syed N. Operant conditioning of aerial respiratory behaviour in *Lymnaea stagnalis*. J Exp Biol 1996;199:683–91.
[19] Syed NI, Bulloch AG, Lukowiak K. In vitro reconstruction of the respiratory central pattern generator of the mollusk Lymnaea. Science 1990;250:282–5.
[20] Syed NI, Ridgway RL, Lukowiak K, Bulloch AGM. Transplantation and functional integration of an identified respiratory interneuron in *Lymnaea stagnalis*. Neuron 1992;8:767–74.
[21] Lukowiak K, Cotter R, Westly J, Ringseis E, Spencer G. Long-term memory of an operantly conditioned respiratory behaviour pattern in *Lymnaea stagnalis*. J Exp Biol 1998;201:877–82.
[22] Lukowiak K, Haque Z, Spencer G, Varshney N, Sangha S, Syed N. Long-term memory survives nerve injury and subsequent regeneration process. Learn Mem 2003;10:44–54.

[23] Lukowiak K, Sangha S, McComb C, Varshney N, Rosenegger D, Sadamoto H, et al. Associative learning and memory in *Lymnaea stagnalis*: how well do they remember? J Exp Biol 2003;206:2097–103.
[24] Lukowiak K, Adatia A, Krygier D, Syed N. Operant conditioning in Lymnaea: evidence for intermediate and long-term memory. Learn Mem 2000;7:140–50.
[25] Sangha S, Scheibenstock A, McComb C, Lukowiak K. Intermediate and long-term memories of associative learning are differentially affected by transcription vs translation blockers in Lymnaea. J Exp Biol 2003;206:1605–13.
[26] Sangha S, Morrow R, Smyth K, Cooke R, Lukowiak K. Cooling blocks ITM and LTM formation and preserves memory. Neurobiol Learn Mem 2003;80: 130–9.
[27] Scheibenstock A, Krygier D, Haque Z, Syed S, Lukowiak K. The soma of RPeD1 must be present for LTM formation of associative learning in Lymnaea. J Neurophysiol 2002;88:1584–91.
[28] Sangha S, Scheibenstock A, Lukowiak K. Reconsolidation of a long- term memory in Lymnaea requires new protein and RNA synthesis and the soma of RPeD1. J Neurosci 2003;23:8034–40.
[29] Sangha S, Scheibenstock A, Morrow R, Lukowiak K. Extinction requires new RNA and protein synthesis and the soma of the cell RPeD1 in *Lymnaea stagnalis*. J Neurosci 2003;23:9842–51.
[30] Sangha S, Scheibenstock A, Martens K, Varshney N, Cooke R, Lukowiak K. Impairing forgetting by preventing new learning and memory. Behav Neurosci 2005;119:787–96.
[31] McComb C, Rosenegger D, Varshney N, Kwok H-Y, Lukowiak K. Operant conditioning of an in vitro CNS-pneumostome preparation of Lymnaea. Neurobiol Learn Mem 2005;84:9–24.
[32] Lattal KM, Radulovic J, Lukowiak K. Extinction: does it or doesn't it? The requirement of altered gene activity and new protein synthesis. Biol Psychiatry 2006;60:344–51.
[33] Orr M, El-Bekai M, Lui M, Watson K, Lukowiak K. Predator detection in Lymnaea. J Exp Biol 2007;210:4150–8.
[34] Boranic M, Poljak-Blazi M. Effect of the crowding stress on hemopoietic colony formation in mice. Exp Hematol 1983;11:873–7.
[35] Holt S. Staying alive in adversity: transcriptome dynamics in the stress-resistant dauer larva. Funct Integr Genom 2006;6:285–99.
[36] Colton H. Some effects of environment on the growth of Lymnaea columella (Say). Proc Natl Acad Sci USA 1908;60:410–48.
[37] Crabb E. Growth of a pond snail. *Lymnaea stagnalis* appressa, as indicated by increase in shell-size. Biol Bull 1929;56:41–63.
[38] Forbes G, Crampton H. The effects of population density upon growth and size in Lymnaea palustris. Biol Bull 1942;83:283–9.
[39] Noland L, Carriker M. Observations on the biology of the snail *Lymnaea stagnalis* appressa during twenty generations in laboratory culture. Am Midl Nat 1946;36:467–93.
[40] Orr M, Lukowiak K. Electrophysiological and behavioural evidence demonstrating that predator detection alters adaptive behaviours in the snail Lymnaea. J Neurosci 2008;28:2726–34.
[41] Orr M, Hittel K, Lukowiak K. Comparing memory-forming capabilities between laboratory-reared and wild Lymnaea. J Exp Biol 2008;211:2807–16.
[42] Orr M, Hittel K, Lukowiak K. 'Different strokes for different folks': geographically isolated strains of *Lymnaea stagnalis* only respond to sympatric predators and have different memory forming capabilities. J Exp Biol 2009;212:2237–47.
[43] Orr M, Hittel K, Lukowiak KS, Han J, Lukowiak K. Differences in LTM-forming capability between geographically different strains of Alberta *Lymnaea stagnalis* are maintained whether they are trained in the lab or in the wild. J Exp Biol 2009;212:3911–8.
[44] Haney J, Lukowiak K. Context learning and the effect of context on memory retrieval in Lymnaea. Learn Mem 2001;8:35–43.
[45] McGaugh J. Memory—a century of consolidation. Science 2000;287(14): 248–51.
[46] Diamond D, Fleshner M, Ingersoll N, Rose G. Psychological stress impairs spatial working memory: relevance to electrophysiological studies of hippocampal function. Behav Neurosci 1996;110:661–72.
[47] Roozendaal B. Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. Neurobiol Learn Mem 2002; 78:578–95.
[48] Woodson J, Macintosh D, Fleshner M, Diamond D. Emotion-induced amnesia in rats: working memory-specific impairment, corticosterone–memory correlation, and fear versus arousal effects on memory. Learn Mem 2003;10:326–36.
[49] Voronezhskaya E, Khabarova M, Nezlin L. Apical sensory neurons mediate developmental retardation induced by conspecific environmental stimuli in freshwater pulmonate snails. Development 2004;131:3671–80.
[50] deCaigny P, Lukowiak K. Crowding, an environmental stressor, blocks long-term memory formation in Lymnaea. J Exp Biol 2008;211:2678–88.
[51] deCaigny P, Lukowiak K. A clash of stressors and LTM formation. Commun Integr Biol 2009;2:1–3.
[52] Boycott AE. The habitats of fresh-water Mollusca in Britain. J Anim Ecol 1936;5:116–86.
[53] Rundle SD, Spicer JI, Coleman RA, Vosper J, Soane J. Environmental calcium modifies induced defences in snails. Proc R Soc B 2004;271:S67–70.
[54] Jeziorski A, Yan ND, Paterson AM, DeSellas AM, Turner MA, Jeffries DS, et al. The widespread threat of calcium decline in fresh waters. Science 2008; 322:1374–7.
[55] Dalesman S, Lukowiak K. Effect of acute exposure to low environmental calcium on respiration and locomotion in *Lymnaea stagnalis* (L.). J Exp Biol 2010;213:1470–1.
[56] Macan TT. Ecology of fresh-water Mollusca in the English Lake District. J Anim Ecol 1950;19:124–46.
[57] Greenaway P. Calcium regulation in the freshwater mollusc. Limnaea stagnalis (L.) (Gastropoda: Pulmonata): 1. The effect of internal and external calcium concentration. J Exp Biol 1971;54:199–214.
[58] Dewith ND, Kok TP, Vanderschors RC. Relationships between apparent net H^+ excretion and Na^+, $Ca2^+$ and Cl^- uptake in the pulmonate freshwater snail *Lymnaea stagnalis*. Comp Biochem Phys A 1987;87:671–5.
[59] Grosell M, Brix KV. High net calcium uptake explains the hypersensitivity of the freshwater pulmonate snail. *Lymnaea stagnalis*, to chronic lead exposure. Aquat Toxicol 2009;91:302–11.
[60] Dewith ND. Evidence for independent regulation of specific ions in hemolymph of *Lymnaea stagnalis* (L). Proc Kon Ned Akad Wet C 1977;80:144–57.
[61] Piggott H, Dussart G. Egg-laying and associated behavioural responses of Lymnaea peregra (Müller) and *Lymnaea stagnalis* (L.) to calcium in their environment. Malacologia 1995;37:13–21.
[62] Mery F, Kawecki TJ. An operating cost of learning in *Drosophila melanogaster*. Anim Behav 2004;68:589–98.