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REVIEW

Environmentally relevant stressors alter memory formation in the pond snail *Lymnaea*

Ken Lukowiak*, Hiroshi Sunada, Morgan Teskey, Kai Lukowiak and Sarah Dalesman[‡]

ABSTRACT

Stress alters adaptive behaviours such as learning and memory. Stressors can either enhance or diminish learning, memory formation and/or memory recall. We focus attention here on how environmentally relevant stressors alter learning, memory and forgetting in the pond snail, *Lymnaea stagnalis*. Operant conditioning of aerial respiration causes associative learning that may lead to long-term memory (LTM) formation. However, individual ecologically relevant stressors, combinations of stressors, and bio-active substances can alter whether or not learning occurs or memory forms. While the behavioural memory phenotype may be similar as a result of exposure to different stressors, how each stressor alters memory formation may occur differently. In addition, when a combination of stressors are presented it is difficult to predict ahead of time what the outcome will be regarding memory formation. Thus, how combinations of stressors act is an emergent property of how the snail perceives the stressors.

KEY WORDS: Operant conditioning, Environmental impact, Memory formation, Long-term memory, Social snails

Introduction

The ability of animals to learn and remember enables them to adapt to environmental changes, and directly affects ‘fitness’. Its common knowledge that stress affects learning and memory. This has been known in the literature since Bacon (Bacon, 1620) and is exemplified in the ‘Yerkes–Dodson Law’ or as we call it the ‘Goldilocks Rule’ (Yerkes and Dodson, 1908) (Fig. 1). Too much or too little stress impedes long-term memory (LTM) formation, while ‘just the right amount’ enhances LTM. Organisms are thought to ‘decide’ to only expend the ‘neuronal cost’ (e.g. altered gene activity and new protein synthesis) necessary to form LTM to ‘relevant’ events. Relevancy is in part determined by the level of stress perceived at the time of learning. Because memory is dynamic, stress can modify memory (e.g. false memory and post-traumatic stress syndrome) (Shors, 2004; Kim and Diamond, 2002; Lukowiak et al., 2003; Lukowiak et al., 2008; Lukowiak et al., 2010). Here we will focus on environmentally relevant stressors that alter memory formation in *Lymnaea*.

Hans Selye, the ‘father of stress research’ stated: ‘Everyone knows what stress is and nobody knows what it is’ (Selye, 1973). Stress is defined here as a state that requires a physiological, psychological or behavioural readjustment or modification in order to maintain the well being of the organism. However, the same stimulus may be perceived as a stressor for one organism but not for

another, or may be perceived as a stressor only at certain times and not at others in the same organism. The effects of stress on learning and memory sometimes yield contradictory results (Shors, 2004). Because of the complexities of the brain and the multitude of behaviours tested, in addition to the different ways in which stressors act, disagreement in the literature on the role that stress plays in learning and memory formation is not too surprising. Using a simpler model system and a relatively simple tractable behaviour, we sought to overcome these problems at both the behavioural and neuronal levels. We also used ecologically relevant stressors in order to obtain an understanding of how learning and memory formation are modified by stressors that snails are likely to encounter in their natural environment (Lukowiak et al., 2010).

We are able to operantly condition, a form of associative learning, aerial respiration in *Lymnaea* (Lukowiak et al., 1996; Lukowiak et al., 1998; Lukowiak et al., 2000). This behaviour is driven by a three-neuron central pattern generator (CPG) whose sufficiency and necessity have been demonstrated (Syed et al., 1990; Syed et al., 1992). As we are dealing with a non-declarative memory, and non-declarative memories are stored in the circuit that mediates the behaviour (Milner et al., 1998), we know where to look in the CNS for the ‘memory trace’. We have found that one of the CPG neurons, Right Pedal Dorsal 1 (RPeD1), is a necessary site for LTM formation, memory reconsolidation, extinction of the initial memory and forgetting (Scheibenstock et al., 2002; Sangha et al., 2003a; Sangha et al., 2003b; Sangha et al., 2003c; Sangha et al., 2005; Lukowiak et al., 2006; Lukowiak et al., 2007). Moreover, the ‘state’ of RPeD1 activity is both predictive and causal as to whether memory will be/has been formed and maintained (Braun et al., 2012).

An important and as yet unexplained observation, and one we will begin to explore here, is that different stressors alter memory formation but not learning (Fig. 2). Notice in these data that learning (i.e. TS2 is significantly less than TS1) is statistically similar in the three cohorts of snails but the two stressors used (crowding and predator scent) alter memory formation differently. We believe that studying why this phenomenon occurs will give us great insight into how stressors alter memory formation and may give valuable clues as to why certain memories may be either more or less difficult to forget. Our working hypothesis is that during the learning phase (i.e. the two training sessions) both ‘memory-making’ and ‘forgetting-making’ processes are activated. Certain stressors favour one process over the other. Whether memory forms and how long it persists is thus dependent on the interaction between the memory and forgetting processes. An understanding of how behaviourally relevant stressors alter LTM formation and/or its strength/persistence may lead to clues as to how the two processes occur in humans and may guide us to possible remedies for disorders such as post-traumatic stress disorder. Such a ‘dual process’ theory has previously been invoked to explain behavioural habituation (Groves and Thompson, 1970; Lukowiak and Jacklet, 1972).

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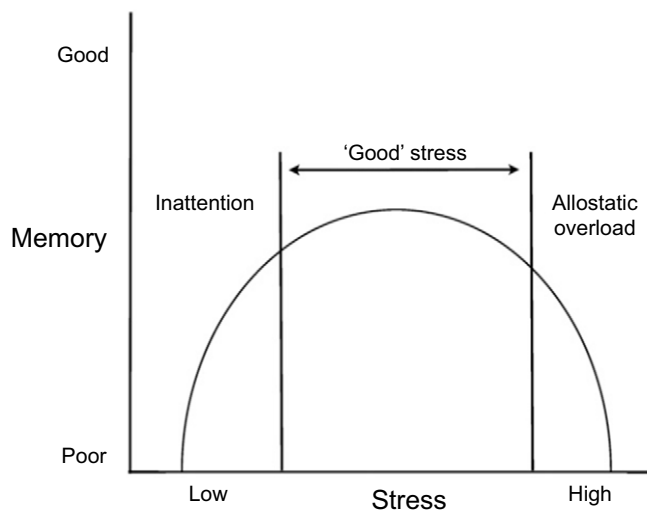


Fig. 1. An idealized version of the Yerkes–Dodson law. On the x-axis we plot the subject's experienced stress level, which runs from 'low' to 'high'. On the y-axis we plot memory spanning the range from 'poor' to 'good'. At low levels of stress there is 'inattention' and this results in a 'poorer' memory, whilst at high levels of stress there is 'allostatic overload' resulting in poor memory. Allostatic overload means that the subject finds it difficult to cope with the stressor and maintain homeostasis. In between the high and low stress levels is the region that we have labelled 'good' stress. We define this 'good' stress level as that which we can cope with, but which is sufficient to keep our attention. Thus, at some levels of stress, memory will be better than at others. This curve can change depending on the age of the individual, their previous history and the difficulty of the task.

A pond's physical environment can readily change (e.g. $[Ca^{2+}]$, temperature changes, etc.) and these changes act as stressors that alter memory. The stressor's effect on adaptive behaviour is dependent on the nature of the stressor; the time the stressor is perceived relative to learning; the specific strain of snail; and importantly, but not often not tested, whether the stressors occur singly or in combination. We can easily manipulate conditions under which snails are maintained and trained to assess how different environmental conditions alter learning, memory and RPeD1 excitability.

Strain differences and stressors

Lymnaea stagnalis collected from ponds in Alberta, the UK and continental Europe (Fig. 3) have given us insight into another not too often researched subject: different strains of the same species have different cognitive abilities. The strain differences described below have been maintained in many generations of snails reared in the lab from freshly collected specimens and have been maintained in those ponds in the wild. Simply put, snails from one pond are 'smarter' than those from another pond, and the ponds can be as close to each other as 500 m (Fig. 4). We define 'smart' as having the ability to form LTM following a single 0.5 h training session. The bulk of memory studies involving *Lymnaea* (Ito et al., 1999; Fulton et al., 2005; Parvez et al., 2006; Sunada et al., 2010) have used laboratory-bred specimens derived from snails originally collected (in the 1950s; >250 generations grown in the lab) from ditches in the Province of Utrecht in The Netherlands. These are referred to here as Dutch snails. In Dutch snails and strains from most of the ponds we sampled (denoted as 'average' snails), a single 0.5 h training session only results in a 3 h memory, which is referred to as intermediate-term memory (ITM). ITM is dependent on new protein synthesis, whereas LTM is dependent on both new protein

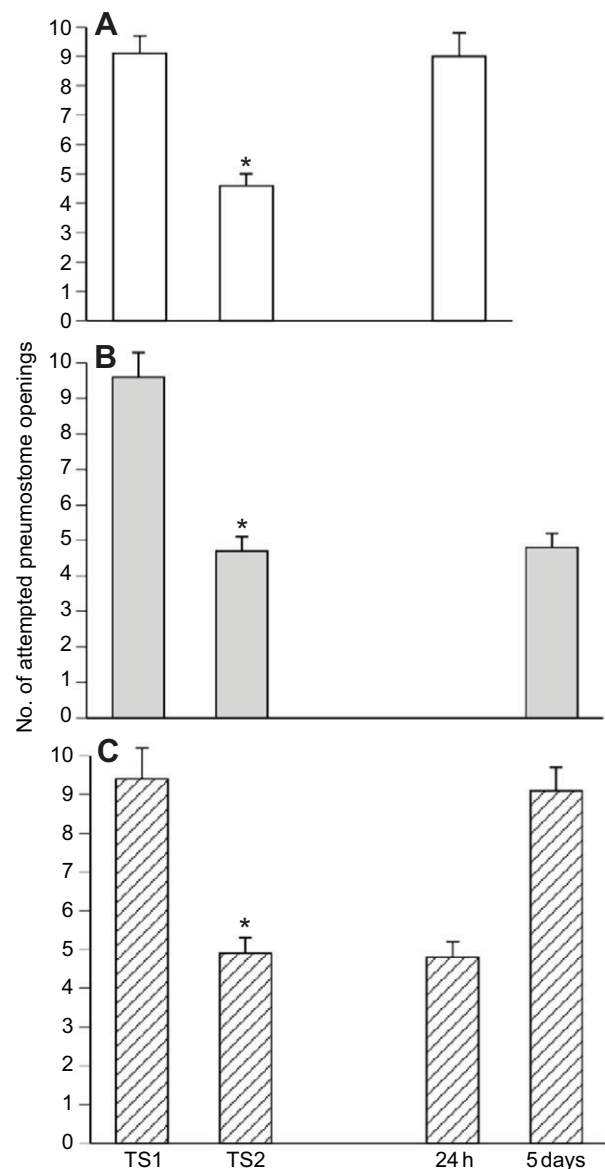


Fig. 2. Similar learning but very different memory. Training of *Lymnaea stagnalis* consisted of two 0.5 h training sessions separated by 1 h (i.e. TS1 and TS2). Three different cohorts of snails ($N=15$ per cohort) all showed learning (i.e. TS2 is significantly less than TS1, asterisk). (A) Crowded for 1 h before training in pond water; (B) trained in pond water following crayfish effluent (CE) exposure; (C) trained in control pond water. There was no LTM following exposure to crowding (A); there was a 5 day memory following exposure to the CE stressor (B); and there was a 24 h memory with training in pond water but not a 5 day memory (C). However, the difference between TS1 and TS2 (i.e. learning) for all three cohorts was the same. There was also no difference in TS1 between the three cohorts. Likewise, there was no difference in TS2 between the three cohorts.

synthesis and altered gene activity (Sangha et al., 2003b; Braun and Lukowiak, 2011). We do not know the cause of this difference in memory-forming capability. We found that RPeD1 is in a different 'state' between the 'smart' and 'average' strains (Braun et al., 2012). In the smart strain the excitability of RPeD1 in naive preparations is significantly less than that in preparations from naive average snails. Interestingly, the level of excitability of RPeD1 in the smart naive preparations is very similar to that seen in a preparation made from an 'average' snail that received a single 0.5 h training session.

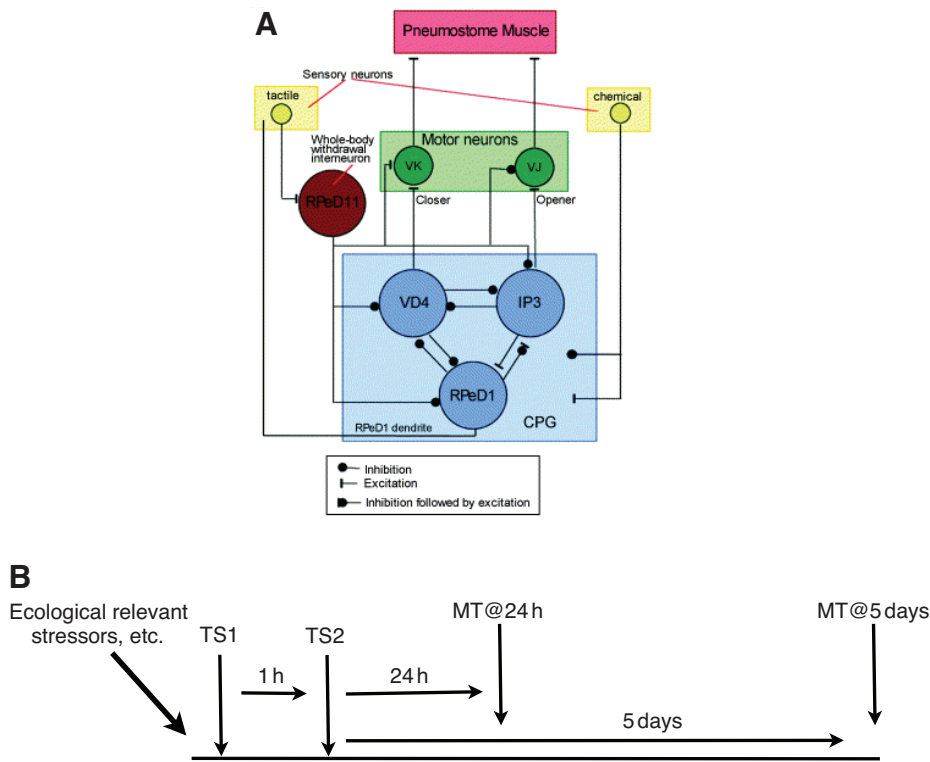


Fig. 3. The central pattern generator (CPG) that drives aerial respiratory behaviour, associated higher order interneurons, sensory inputs and motor neuron outputs. (A) The three CPG neurons (VD4, IP3 and RPeD1) have been shown to be both necessary and sufficient to drive aerial respiratory behaviour. RPeD1 has been demonstrated to be a necessary site for long-term memory (LTM) formation, reconsolidation, extinction and forgetting. Moreover, we have recently shown that the 'state' of RPeD1 activity predicts the ability of the snail to make LTM. Rhythmogenesis is an emergent property of this network. Input from the periphery plays a major role in determining the level of spontaneous activity of neurons such as RPeD1. (B) A time line showing the experimental plan when 'stress' studies are performed on the ability of *Lymnaea* to learn and form LTM. Typically, the stressor of a bio-active agent is presented to the snail before training (one or two training sessions, TS). Whether LTM is formed and for how long is then determined by testing for memory at some later time point (memory test, MT; here, 24 h and 5 days).

In other words, it appears that RPeD1 in smart snails is 'primed' for memory formation. Our present working and as yet unsubstantiated hypothesis is that 'smart' snails are more easily stressed than 'average' snails.

Stressful stimuli

There are a number of factors that are important in the life of a snail, changes that could lead to stressful times. *Lymnaea*, to live long and prosper, requires adequate food, a good source of calcium, which it absorbs directly from the water to grow its shell (see below), and the ability to detect predators (Dalesman and Lukowiak, 2010). Restrictions of either food or calcium lead to stunting and reduced reproduction. Thus, food or decreased calcium availability act as environmental stressors. Crowded conditions may increase competition for resources. Conversely, as a preferentially out-crossing hermaphrodite, isolation and therefore not being able to find a mate is also not ideal. Consequently, either crowding or isolation could act as social stressors.

Typically, *Lymnaea* are found living in slow flowing or stagnant shallow water bodies. In temperate regions these habitats may be subject to rapid temperature fluctuations. During a summer day in Alberta with the sun shining, water temperature may rapidly rise in a shallow pond, and then with frost overnight could drop equally rapidly to close to freezing. These rapid temperature fluctuations are not uncommon, and may act as a stressor.

Resource restriction

Memory formation is costly (Barnard et al., 2006; Burns et al., 2011); hence, if energy intake is restricted, LTM formation might be blocked in order to conserve energy. We food-deprived *Lymnaea* for 5 days prior to training and these snails had no problem forming LTM (Haney and Lukowiak, 2001). Thus, 5 days without food was not sufficient to block LTM. However, if food-deprived animals are trained in the presence of a food smell, snails no longer learn nor

form memory. Thus, detecting food when food deprived blocks both learning and memory. We speculate that when a food source is detected it acts as a stressor when the snail has previously been starved. It is important to remember that the 'state' of the organism in large measure determines how it perceives a stimulus as a stressor. The perception of the stimulus, rather than the stimulus itself, acts as the stressor. We will examine in the future how food detection acts as a stressor when the snail has been food deprived. Presumably, sensory inputs to respiratory CPG neurons alter their state of activity such that LTM formation is compromised.

Lymnaea also require calcium in pond water. *Lymnaea* absorb calcium directly from the water and not from food sources. Populations of this species are generally found where environmental calcium is $\geq 20 \text{ mg l}^{-1}$ (Boycott, 1936; Young, 1975; Madsen, 1987). We hypothesized that at $20 \text{ mg l}^{-1} [\text{Ca}^{2+}]$, snails would be stressed; we found that following short (~1 h) periods of exposure to $20 \text{ mg l}^{-1} [\text{Ca}^{2+}]$, *Lymnaea* no longer formed LTM, though they still demonstrated ITM (Fig. 5) (Dalesman et al., 2011a). There was no alteration in the electrophysiological activity of RPeD1 in naive animals relative to calcium availability, but significant differences between calcium environments were apparent in RPeD1 24 h following training. In the $80 \text{ mg l}^{-1} [\text{Ca}^{2+}]$ 'standard' environment, RPeD1 activity was significantly depressed relative to the naive state; in $20 \text{ mg l}^{-1} [\text{Ca}^{2+}]$, only a partial change in activity occurred.

The osphradium is a chemosensory organ that snails 'sniff' the water with, and it has electrophysiological responses to a wide range of chemicals (Wedemeyer and Schild, 1995; Kamardin et al., 2001; Nezhlin, 2004). We severed the osphradial ganglion connecting the osphradium to the CNS, and compared the response of osphradially cut snails with that of sham-operated animals to training following exposure to low environmental calcium (Dalesman et al., 2011b). *Lymnaea* with a cut osphradial nerve exposed to low environmental calcium prior to training formed LTM, equivalent to animals maintained in our standard calcium pond water (Fig. 6). However,

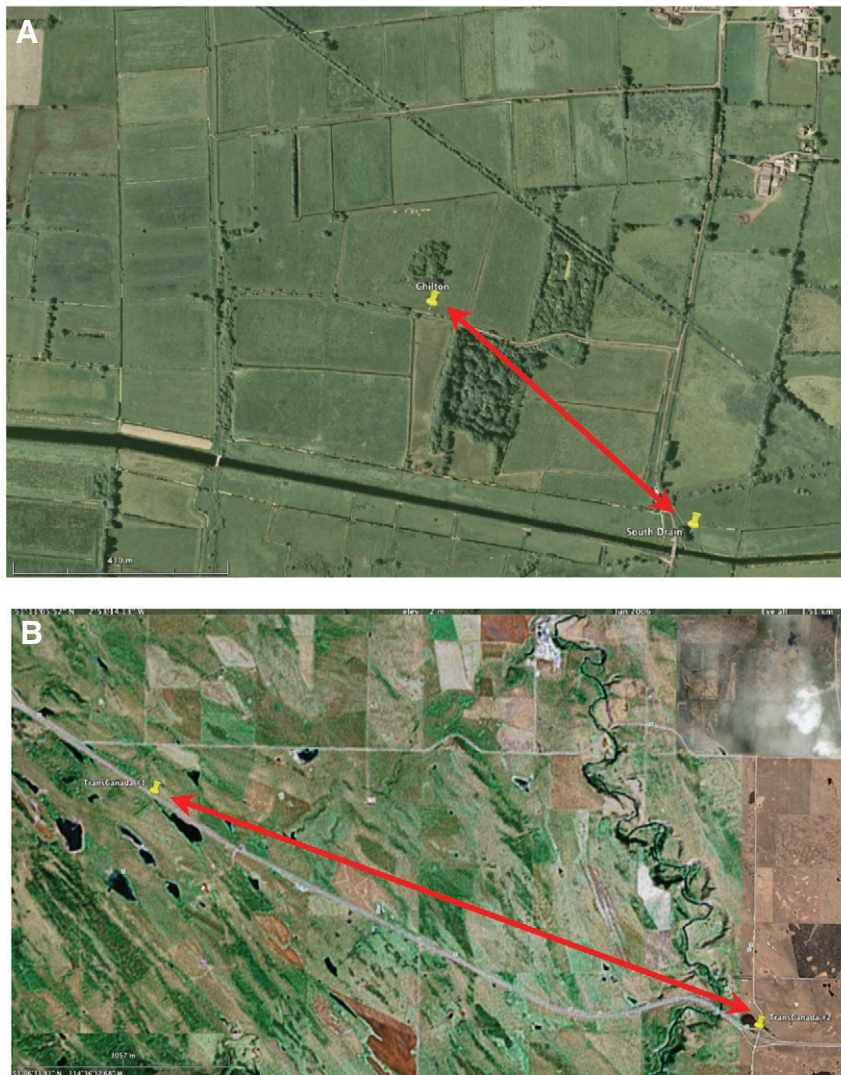


Fig. 4. Distances between ponds that have been sampled over a number of years that contain ‘smart’ and ‘average’ snails. (A) A map (Google Maps) showing two ponds in the Somerset Levels of Southwest England. Chilton Moor is separated from South Drain by ~500 m. Both ponds are ~2 m above sea level. (B) A map (Google Maps) showing ponds labelled TC1 and TC2. They are both located along the Trans-Canada highway west of the city of Calgary at an altitude of ~1300 m above sea level. The distance between the two ponds is ~9 km. The two ponds are in the same watershed (Jumping Pound Creek), which flows into the Bow River and ultimately into Hudson’s Bay.

LTM was blocked following exposure to low environmental calcium in our sham-operated animals. Thus, the osphradial input mediates the memory-blocking effect of low environmental calcium in *Lymnaea*. These data also show that the stress is modulated via sensing the external environment and not due to changes in internal Ca^{2+} homeostasis. While we see clear changes in RPeD1 activity with stress predator cues (Orr et al., 2007; Orr et al., 2008; Orr and Lukowiak, 2008; Martens et al., 2007), mediated by osphradial input, no significant change occurs in RPeD1 activity in naive animals following exposure to low calcium (Dalesman et al., 2011a). Clearly, the effect that low calcium has on the CNS is modulated outside the CPG controlling aerial respiratory behaviour.

Crowding and isolation

Lymnaea population density in ponds fluctuates widely within and between years. Often in a pond, during one week we struggle to find a solitary individual, whereas less than a month later a single net sweep results in collecting tens of animals. Additionally, the distribution of snails within a pond varies greatly such that we find many individuals to no individuals within a metre or so. Thus, snails can experience either crowded or solitary conditions in a pond and the ‘social status’ of an individual can easily vary. Crowded conditions reduce reproduction and survival in *Lymnaea* (Noland

and Carriker, 1946; McDonald, 1969; Brown, 1979; Janse et al., 1988), suggesting that crowding is a stressor. Isolation of individuals significantly alters their copulatory behaviour; following a week of isolation, *Lymnaea* will preferentially perform in the male role during mating (De Boer et al., 1997).

We assessed the effects of crowding (20 snails per 100 ml) on LTM formation in Dutch snails. Crowding them either immediately before or after operant conditioning blocked LTM formation; but they still learned and formed ITM (De Caigny and Lukowiak, 2008a). Crowding is thus incompatible with the gene transcription required for LTM formation (Sangha et al., 2003b). Crowding, however, did not block memory recall. We also found that osphradial input was not necessary for crowding to block LTM (Dalesman et al., 2011b).

We also chose to look at isolation, assessing whether an acute period of isolation alters memory. Snails were isolated in perforated containers, where several such containers were maintained in the same aquaria. Therefore, there was the potential for waterborne chemical cues to pass between the animals, but they were unable to make physical contact. These animals were trained and tested in isolation. Contrary to our predictions in Dutch snails, we found that social isolation, whether during maintenance and training/testing or during training/testing alone, did not alter learning and memory in

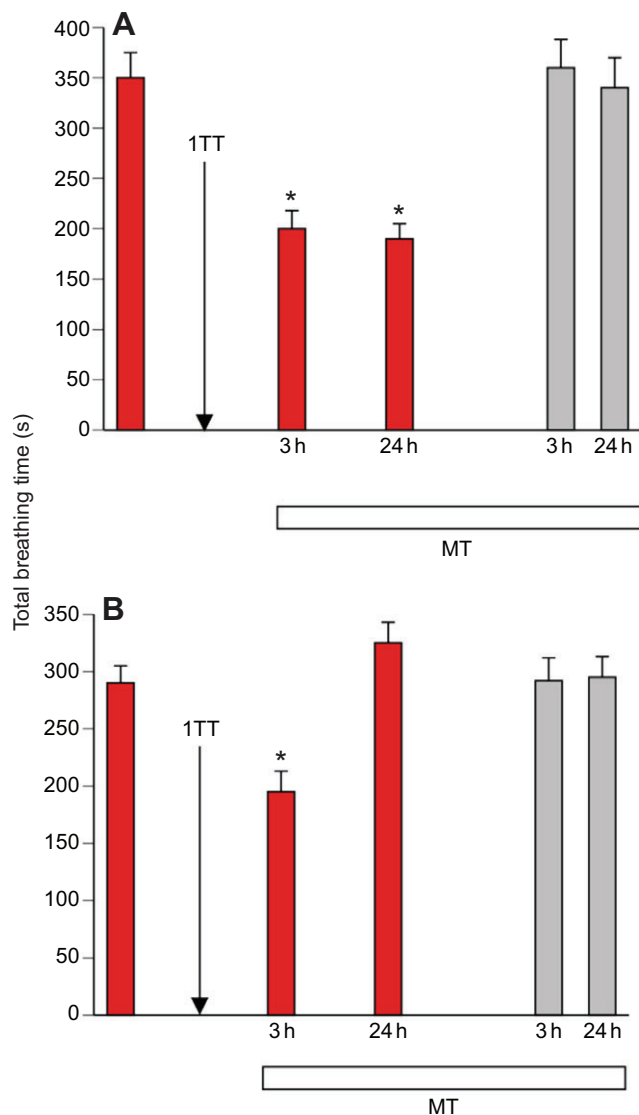


Fig. 5. Memory following a 1 week exposure to normal or low calcium pond water environments. Mean total breathing time in standard (A; 80 mg l⁻¹) and low (B; 20 mg l⁻¹) environmental calcium prior to and following one-trial conditioning (1TT, arrow). *Memory present ($P < 0.05$). The yoked control data (grey) show that only contingent pairing of the pneumostome opening attempt results in memory formation. As can be seen in both A and B, memory was present at 3 h, while LTM was only present in normal calcium pond water (A).

Lymnaea (Dalesman and Lukowiak, 2011b). Thus, social isolation did not alter memory. However, we later discovered that the effects of isolation are strain and context dependent.

Interaction between stressors

Typically, the effects of stress on learning and memory are assessed using a single stressor. Indeed, much of our work only addressed the effects of a single stressor on learning and memory. However, in 'real life', we face multiple stressors. We investigated how *Lymnaea* respond to multiple stressors. Could we successfully predict how different stressors will interact to alter learning and memory based on their individual effects? The answer is that the interaction of stressors on memory is an emergent property (i.e. basically unpredictable).

We first tested a combination of crayfish effluent (CE) and crowding. When experienced as a single stressor, CE enhances LTM (Orr and Lukowiak, 2008) whereas crowding blocks LTM (De Caigny and Lukowiak, 2008a). In Dutch snails, a single 0.5 h training session in CE results in LTM, whereas in control conditions only ITM results (Orr and Lukowiak, 2008). However, crowding *Lymnaea* immediately prior to 0.5 h training in CE negated the enhancing effect of CE (i.e. LTM was not formed) (De Caigny and Lukowiak, 2008b). Thus, crowding effectively 'trumped' the effects of CE. More recent work indicates that the interaction between CE and crowding may not be this simple (i.e. crowding may not always 'block' the effects of CE), but further work is required to present a clear picture of how these two stressors interact.

A second stressor that blocks memory formation is low calcium (20 mg l⁻¹) pond water. Similarly to crowding, this stressor blocks LTM formation, but snails are still able to learn and form ITM (Dalesman et al., 2011a; Knezevic et al., 2011). When we operantly trained animals that were held in low calcium in the presence of CE, they were subsequently able to form LTM (Dalesman and Lukowiak, 2011a), suggesting that training in CE may prevent the memory-blocking effects of a low calcium environment. However, CE has previously been shown to extend LTM retention. In control conditions, snails normally form memory lasting 24–48 h following two 0.5 h training sessions, but if exposed to CE during this training regime, LTM lasts for 8 days (Orr and Lukowiak, 2008). To confirm whether CE was preventing the effects of low calcium on memory, we also tested for LTM 72 h following training in CE after exposing snails to low calcium. We found that snails no longer showed LTM 72 h following training, when we would expect them to still show strong memory retention (Dalesman and Lukowiak, 2011a). In fact, in the presence of both stressors the snails now showed an identical memory phenotype to the one they showed under control conditions, so the effects of each stressor appear to have effectively cancelled each other out. Sensory input for modulation of memory phenotype comes via the osphradium for both low calcium conditions (Dalesman et al., 2011b) and CE (El-Han et al., 2010; Karnik et al., 2012a). We consider that it is likely that these two inputs when processed in the CNS result in an identical behavioural phenotype to that of control trained animals.

A third environmental factor we decided to consider in combination with other environmental stressors is social isolation. This was particularly interesting to us as we were somewhat surprised that this 'stressor' did not alter memory formation in control conditions (Dalesman and Lukowiak, 2011b), considering the strong effect it has on copulatory behaviour (De Boer et al., 1997). We decided to test how socially isolated *Lymnaea* formed LTM following operant conditioning in the presence of two additional environmental stressors: low calcium and CE (Fig. 7). Socially isolated snails trained in the presence of CE formed LTM at 24 h, showing no difference relative to grouped animals trained in the presence of this stressor. However, when we isolated *Lymnaea* in low calcium, which normally blocks LTM, they now formed LTM (Dalesman and Lukowiak, 2012). Whilst the finding that social isolation can actually enhance memory in low calcium conditions seems surprising, we think this may be due to a change in reproductive behaviour. Adult *Lymnaea* normally produce 100–300 eggs per week (Noland and Carriker, 1946; Nichols et al., 1971; Brown, 1979), each of which is provisioned with its own small amount of calcium (Taylor, 1973; Ebanks et al., 2010). However, switching energy investment primarily towards male reproductive output in isolation (De Boer et al., 1997) may significantly reduce the calcium requirements of *Lymnaea*, and reduce the stress

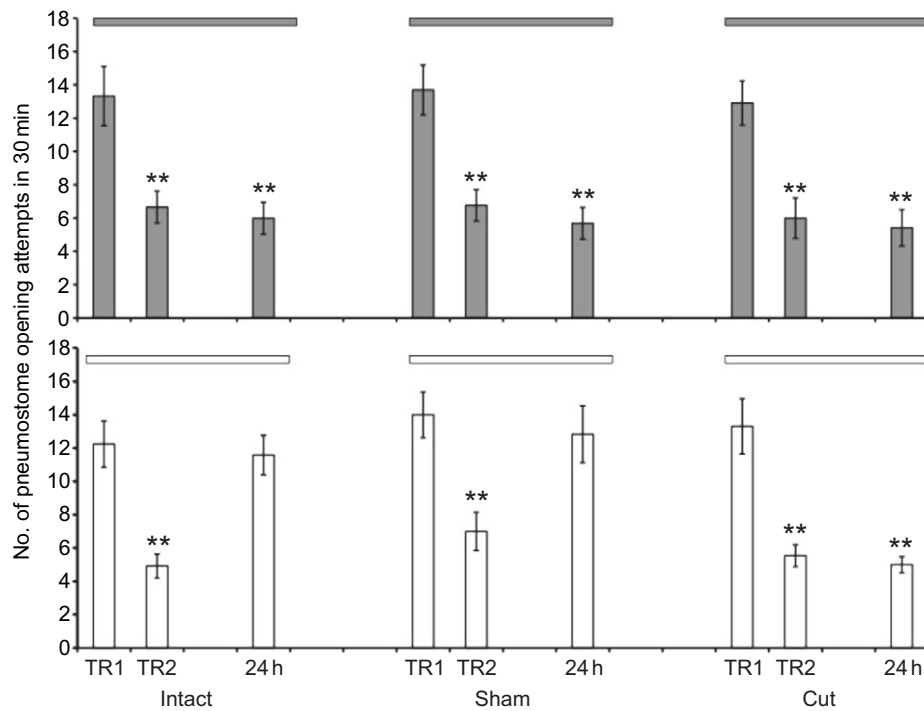


Fig. 6. An intact osphradial nerve is necessary for low calcium pond water to block LTM formation. Snails were intact, sham operated or had a severed osphradial nerve. The figure shows the number of pneumostome opening attempts during the two training sessions (TR1 and TR2) and the memory test at 24 h, following exposure to either low (20 mg l⁻¹; white bars, bottom graphs) or standard (80 mg l⁻¹; grey bars, top graphs) environmental calcium for 1 week prior to tests and throughout the training and testing period. Horizontal bars above the columns indicate the conditions in which snails were maintained in their eumoxic aquaria (grey, normal; white, low calcium). Data are means ± s.e.m. **Significantly different from TR1 within the training group (paired *t*-test, *P*<0.01). Data replotted with permission (Dalesman et al., 2011b).

associated with being held in low calcium conditions, allowing these animals to form LTM. An even greater surprise was that when we trained isolated snails that had been held in low calcium conditions in the presence of CE (which normally enhances memory formation), whilst the animals were able to demonstrate learning, LTM was blocked (Fig. 7). When conditions are so stressful, *Lymnaea* are simply too stressed to pay attention to training, as they did when they were hungry and trained in the presence of a food odour.

Our data present evidence to support negative, neutral and enhancing effects of social isolation on memory formation following identical training, showing that stressor effects are highly dependent on ‘context’ (Dalesman and Lukowiak, 2011b). Thus, inconsistencies in the literature on the effects of isolation on cognition may be due to changes in other environmental variables that are often not described. The data also show that when stressors are experienced in combination, the outcome is not easily predicted. This work provides an intriguing avenue for future exploration, and

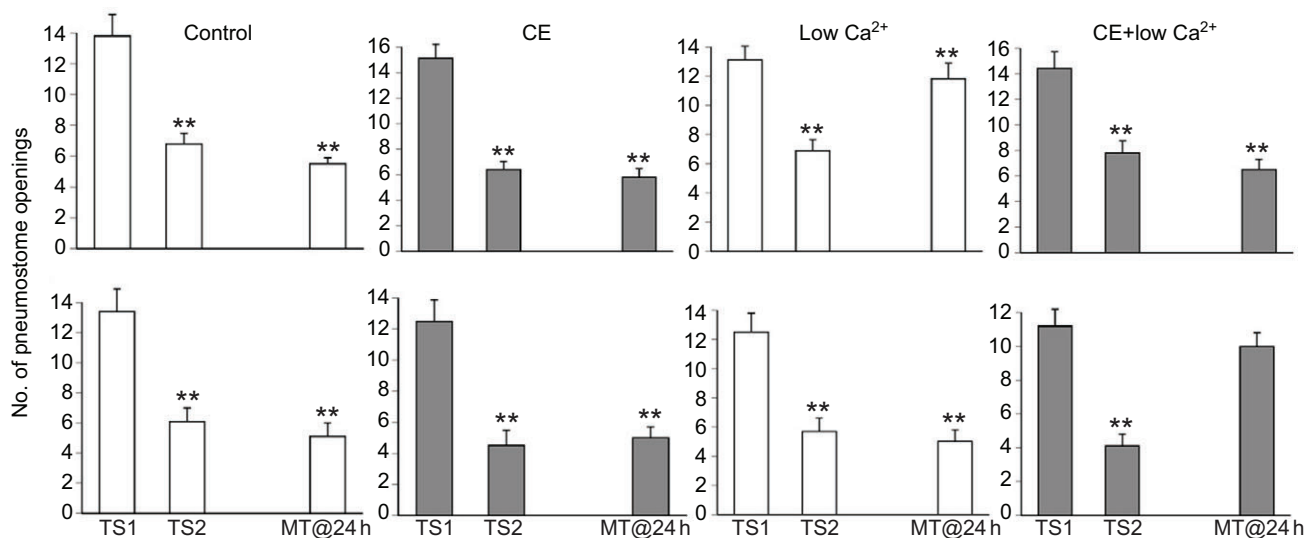


Fig. 7. Social stress in combination with other stressors has differing effects on LTM formation. Top, snails that were not socially isolated; bottom, snails that were socially isolated. Plotted are the mean and s.e.m. number of attempted pneumostome openings. In the control group, it can be seen that social isolation has no effect on memory formation. In the crayfish effluent (CE) group it can be seen that social isolation also has no effect on the enhancing effect of CE on LTM formation. In the low Ca²⁺ group, social isolation alters the effect of low environmental calcium on LTM formation. Low Ca²⁺ blocks LTM formation when snails are in a group, but this blocking of LTM is not seen when snails are socially isolated. We could not have predicted this outcome before doing the experiment. Finally, the CE+low Ca²⁺ group demonstrates the effect of low Ca²⁺ and CE exposure on socially isolated snails. As can be seen, when snails are not isolated, CE ‘overcomes’ the effect of low Ca²⁺ on LTM formation. However, when socially isolated, the effects of low Ca²⁺ and CE result in the suppression of memory formation. ***P*<0.01. Data replotted with permission (Dalesman and Lukowiak, 2011b).

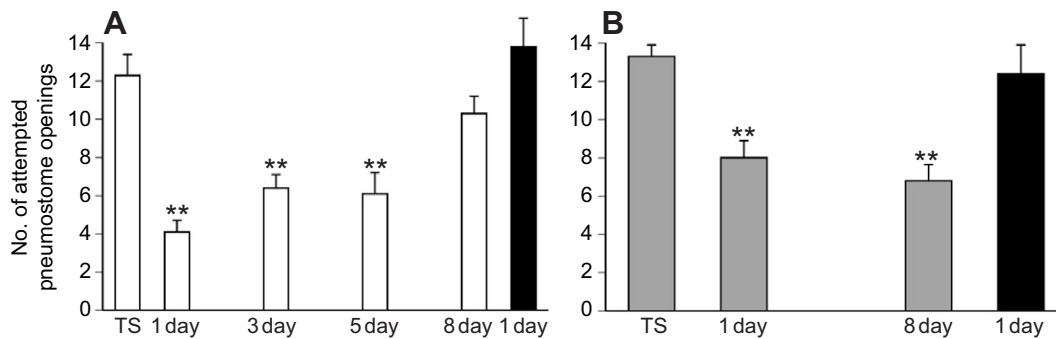


Fig. 8. Smart snails and CE. The mean and s.e.m. number of attempted pneumostome openings. (A) In smart snails obtained from a pond in the Somerset Levels in the UK, a single 0.5 h training session results in a memory that persists for 5 but not 8 days. In lab-bred snails, a single 0.5 h training session results in a memory that only persists for up to 3 h (not shown). (B) However, predator detection (CE) in the smart snails results in enhanced LTM formation as now memory persists for over 8 days. In A and B we present data showing that the yoked control procedure (black bars) does not result in LTM when tested at 24 h. ** $P < 0.01$. Data in A replotted with permission (Dalesman and Lukowiak, 2012).

we already have a wealth of environmental stress combinations to test. For example, does food restriction, which on its own had little effect, alter the way in which a snail responds to other stressors (i.e. similar to social isolation), or can stressors with identical directional effects on memory retention when presented alone have cumulative effects? Importantly, this raises our awareness, and hopefully that of others reading this work, in detailing other environmental factors experienced by the animal when testing for stress effects.

Having identified populations in both the UK and Canada that differ in memory-forming ability, we were interested in how these ‘smart’ snails would respond to environmental stressors relative to the ‘lab’ snails. For example, we had not shown that predator detection extended memory retention beyond that seen in control conditions in the same way as in the lab-reared snails (Orr et al., 2009a; Orr et al., 2009b; Dalesman et al., 2011b). It was possible that ‘smart’ *Lymnaea* were already at their maximum ability to form LTM, whereas the Dutch snails were more plastic, only exhibiting maximum LTM formation in the presence of a stressor such as CE. We found that while ‘smart’ snails exhibit LTM lasting 5 but not 8 days in control conditions (Dalesman and Lukowiak, 2012), when we trained them in predator cues (CE), we could extend this memory even further, such that it lasted at least 8 days (Fig. 8). Therefore, the ‘smart’ snails are still able to show plasticity in LTM formation, despite starting in a primed state (Braun et al., 2012).

It seems that all snail populations tested respond to ecologically relevant stressors in a similar directional pattern. However, further work is required to assess whether this is the case for all stressors. We have shown that regardless of whether snails are ‘smart’ or ‘average’ and whether they were obtained in North America or Europe, they respond in a similar direction to a stressor. This indicates that whatever mechanisms are controlling LTM formation, they are highly conserved across the *Lymnaea stagnalis* strains, showing similar patterns on both broad and narrow geographic scales.

Competing interests

The authors declare no competing financial interests.

Author contributions

All authors have contributed to the design and performing of the experiments. Ken Lukowiak wrote the initial manuscript, but all authors made accepted suggested changes.

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References

- Bacon, F. (1620). *The New Organon*. New York, NY: Bobbs-Merrill.
- Barnard, C. J., Collins, S. A., Daisley, J. N. and Behnke, J. M. (2006). Odour learning and immunity costs in mice. *Behav. Processes* **72**, 74–83.
- Boycott, A. E. (1936). The habitats of fresh-water Mollusca in Britain. *J. Anim. Ecol.* **5**, 116–186.
- Braun, M. H. and Lukowiak, K. (2011). Intermediate and long-term memory are different at the neuronal level in *Lymnaea stagnalis* (L.). *Neurobiol. Learn. Mem.* **96**, 403–416.
- Braun, M. H., Lukowiak, K., Karnik, V. and Lukowiak, K. (2012). Differences in neuronal activity explain differences in memory forming abilities of different populations of *Lymnaea stagnalis*. *Neurobiol. Learn. Mem.* **97**, 173–182.
- Brown, K. M. (1979). The adaptive demography of four freshwater pulmonate snails. *Evolution* **33**, 417–432.
- Burns, J. G., Foucaud, J. and Mery, F. (2011) Costs of memory: lessons from ‘mini’ brains. *Proc. R. Soc. Lond. B* **278**, 923–929.
- Dalesman, S. and Lukowiak, K. (2010). Effect of acute exposure to low environmental calcium on respiration and locomotion in *Lymnaea stagnalis* (L.). *J. Exp. Biol.* **213**, 1471–1476.
- Dalesman, S. and Lukowiak, K. (2011a). Interaction between environmental stressors mediated via the same sensory pathway. *Commun. Integr. Biol.* **4**, 717–719.
- Dalesman, S. and Lukowiak, K. (2011b). Social snails: the effect of social isolation on cognition is dependent on environmental context. *J. Exp. Biol.* **214**, 4179–4185.
- Dalesman, S. and Lukowiak, K. (2012). How stress alters memory in ‘smart’ snails. *PLoS ONE* **7**, e32334.
- Dalesman, S., Braun, M. H. and Lukowiak, K. (2011a). Low environmental calcium blocks long-term memory formation in a freshwater pulmonate snail. *Neurobiol. Learn. Mem.* **95**, 393–403.
- Dalesman, S., Karnik, V. and Lukowiak, K. (2011b). Sensory mediation of memory blocking stressors in the pond snail *Lymnaea stagnalis*. *J. Exp. Biol.* **214**, 2528–2533.
- Dalesman, S., Rundle, S. D. and Lukowiak, K. (2011c). Microgeographic variability in long-term memory formation in the pond snail, *Lymnaea stagnalis*. *Anim. Behav.* **82**, 311–319.
- De Boer, P., Jansen, R. F., Koene, J. M. and Maat, A. (1997). Nervous control of male sexual drive in the hermaphroditic snail. *J. Exp. Biol.* **200**, 941–951.
- De Caigny, P. and Lukowiak, K. (2008a). A clash of stressors and LTM formation. *Commun. Integr. Biol.* **1**, 125–127.
- De Caigny, P. and Lukowiak, K. (2008b). Crowding, an environmental stressor, blocks long-term memory formation in *Lymnaea*. *J. Exp. Biol.* **211**, 2678–2688.
- Ebanks, S. C., O’Donnell, M. J. and Grosell, M. (2010). Characterization of mechanisms for Ca^{2+} and $\text{HCO}_3^-/\text{CO}_3^{2-}$ acquisition for shell formation in embryos of the freshwater common pond snail *Lymnaea stagnalis*. *J. Exp. Biol.* **213**, 4092–4098.
- Fulton, D., Kemenes, I., Andrew, R. J. and Benjamin, P. R. (2005). A single time-window for protein synthesis-dependent long-term memory formation after one-trial appetitive conditioning. *Eur. J. Neurosci.* **21**, 1347–1358.
- Groves, P. M. and Thompson, R. F. (1970). Habituation: a dual-process theory. *Psychol. Rev.* **77**, 419–450.
- Haney, J. and Lukowiak, K. (2001). Context learning and the effect of context on memory retrieval in *Lymnaea*. *Learn. Mem.* **8**, 35–43.
- Il-Han, J., Janes, T. and Lukowiak, K. (2010). The role of serotonin in the enhancement of long-term memory resulting from predator detection in *Lymnaea*. *J. Exp. Biol.* **213**, 3603–3614.
- Ito, E., Kobayashi, S., Kojima, S., Sadamoto, H. and Hatakeyama, D. (1999). Associative learning in the pond snail, *Lymnaea stagnalis*. *Zoolog. Sci.* **16**, 711–723.

- Janse, C., Slob, W., Popelier, C. M. and Vogelaar, J. W. (1988). Survival characteristics of the mollusc *Lymnaea stagnalis* under constant culture conditions: effects of aging and disease. *Mech. Ageing Dev.* **42**, 263-274.
- Kamardin, N. N., Shalanki, Y., Rozha, K. S. and Nozdrachev, A. D. (2001). Studies of chemoreceptor perception in mollusks. *Neurosci. Behav. Physiol.* **31**, 227-235.
- Karnik, V., Braun, M. H., Dalesman, S. and Lukowiak, K. (2012a). Sensory input from the osphradium modulates the response to memory-enhancing stressors in *Lymnaea stagnalis*. *J. Exp. Biol.* **215**, 536-542.
- Karnik, V., Dalesman, S. and Lukowiak, K. (2012b). Input from a chemosensory organ, the osphradium, does not mediate aerial respiration in *Lymnaea stagnalis*. *Aquat. Biol.* **15**, 167-173.
- Kim, J. J. and Diamond, D. M. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Nat. Rev. Neurosci.* **3**, 453-462.
- Knezevic, B., Dalesman, S., Karnik, V., Byzitter, J. and Lukowiak, K. (2011). Low external environmental calcium levels prevent forgetting in *Lymnaea*. *J. Exp. Biol.* **214**, 2118-2124.
- Lukowiak, K. and Jacklet, J. W. (1972). Habituation and dishabituation: interactions between peripheral and central nervous systems in *Aplysia*. *Science* **178**, 1306-1308.
- Lukowiak, K., Ringseis, E., Spencer, G., Wildering, W. and Syed, N. (1996). Operant conditioning of aerial respiratory behaviour in *Lymnaea stagnalis*. *J. Exp. Biol.* **199**, 683-691.
- Lukowiak, K., Cotter, R., Westly, J., Ringseis, E., Spencer, G. and Syed, N. (1998). Long-term memory of an operantly conditioned respiratory behaviour pattern in *Lymnaea stagnalis*. *J. Exp. Biol.* **201**, 877-882.
- Lukowiak, K., Adatia, N., Krygier, D. and Syed, N. (2000). Operant conditioning in *Lymnaea*: evidence for intermediate- and long-term memory. *Learn. Mem.* **7**, 140-150.
- Lukowiak, K., Sangha, S., Scheibenstock, A., Parvez, K., McComb, C., Rosenegger, D., Varshney, N. and Sadamoto, H. (2003). A molluscan model system in the search for the engram. *J. Physiol. Paris* **97**, 69-76.
- Lukowiak, K., Martens, K., Orr, M., Parvez, K., Rosenegger, D. and Sangha, S. (2006). Modulation of aerial respiratory behaviour in a pond snail. *Respir. Physiol. Neurobiol.* **154**, 61-72.
- Lukowiak, K., Fras, M., Smyth, K., Wong, C. and Hittel, K. (2007). Reconsolidation and memory infidelity in *Lymnaea*. *Neurobiol. Learn. Mem.* **87**, 547-560.
- Lukowiak, K., Martens, K., Rosenegger, D., Browning, K., De Caigny, P. and Orr, M. (2008). The perception of stress alters adaptive behaviours in *Lymnaea stagnalis*. *J. Exp. Biol.* **211**, 1747-1756.
- Lukowiak, K., Orr, M., De Caigny, P., Lukowiak, K. S., Rosenegger, D., Han, J. I. and Dalesman, S. (2010). Ecologically relevant stressors modify long-term memory formation in a model system. *Behav. Brain Res.* **214**, 18-24.
- Madsen, H. (1987). Effect of calcium concentration on growth and egg laying of *Helisoma duryi*, *Bulinus africanus* and *Bulinus truncatus* (Gastropoda: Planorbidae). *J. Appl. Ecol.* **24**, 823-836.
- Martens, K. R., De Caigny, P., Parvez, K., Amarell, M., Wong, C. and Lukowiak, K. (2007). Stressful stimuli modulate memory formation in *Lymnaea stagnalis*. *Neurobiol. Learn. Mem.* **87**, 391-403.
- McDonald, S. L. C. (1969). The biology of *Lymnaea stagnalis* (L.) (Gastropoda: Pulmonata). *Sterkiana* **36**, 1-17.
- Milner, B., Squire, L. R. and Kandel, E. R. (1998). Cognitive neuroscience and the study of memory. *Neuron* **20**, 445-468.
- Nezlin, L. P. (2004). [Structure and electrophysiological properties of bursting neurosecretory cells in a peripheral sensory ganglion of the pond snail *Lymnaea stagnalis*]. *Zh. Vyssh. Nerv. Deiat. Im. I P Pavlova* **54**, 632-637.
- Nichols, D., Cooke, J. and Whiteley, D. (1971) *The Oxford Book of Invertebrates*. Oxford: Oxford University Press.
- Noland, L. E. and Carriker, M. R. (1946). Observations on the biology of the snail *Lymnaea stagnalis* appressa during twenty generations in laboratory culture. *Am. Midl. Nat.* **36**, 467-493.
- Orr, M. V. and Lukowiak, K. (2008). Electrophysiological and behavioral evidence demonstrating that predator detection alters adaptive behaviors in the snail *Lymnaea*. *J. Neurosci.* **28**, 2726-2734.
- Orr, M. V., El-Bekai, M., Lui, M., Watson, K. and Lukowiak, K. (2007). Predator detection in *Lymnaea stagnalis*. *J. Exp. Biol.* **210**, 4150-4158.
- Orr, M. V., Hittel, K. and Lukowiak, K. (2008). Comparing memory-forming capabilities between laboratory-reared and wild *Lymnaea*: learning in the wild, a heritable component of snail memory. *J. Exp. Biol.* **211**, 2807-2816.
- Orr, M. V., Hittel, K. and Lukowiak, K. (2009a). '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.* **212**, 2237-2247.
- Orr, M., Hittel, K., Lukowiak, K. S., Han, J. and Lukowiak, K. (2009b). 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.* **212**, 3911-3918.
- Parvez, K., Rosenegger, D., Martens, K., Orr, M. and Lukowiak, K. (2006). Canadian Association of Neurosciences Review: learning at a snail's pace. *Can. J. Neuro. Sci.* **33**, 347-356.
- Sangha, S., Scheibenstock, A. and Lukowiak, K. (2003a). Reconsolidation of a long-term memory in *Lymnaea* requires new protein and RNA synthesis and the soma of right pedal dorsal 1. *J. Neurosci.* **23**, 8034-8040.
- Sangha, S., Scheibenstock, A., McComb, C. and Lukowiak, K. (2003b). Intermediate and long-term memories of associative learning are differentially affected by transcription versus translation blockers in *Lymnaea*. *J. Exp. Biol.* **206**, 1605-1613.
- Sangha, S., Scheibenstock, A., Morrow, R. and Lukowiak, K. (2003c). Extinction requires new RNA and protein synthesis and the soma of the cell right pedal dorsal 1 in *Lymnaea stagnalis*. *J. Neurosci.* **23**, 9842-9851.
- Sangha, S., Scheibenstock, A., Martens, K., Varshney, N., Cooke, R. and Lukowiak, K. (2005). Impairing forgetting by preventing new learning and memory. *Behav. Neurosci.* **119**, 787-796.
- Scheibenstock, A., Krygier, D., Haque, Z., Syed, N. and Lukowiak, K. (2002). The Soma of RPeD1 must be present for long-term memory formation of associative learning in *Lymnaea*. *J. Neurophysiol.* **88**, 1584-1591.
- Selye, H. (1973). The evolution of the stress concept. *Am. Sci.* **61**, 692-699.
- Shors, T. J. (2004). Learning during stressful times. *Learn. Mem.* **11**, 137-144.
- Sunada, H., Sakaguchi, T., Horikoshi, T., Lukowiak, K. and Sakakibara, M. (2010). The shadow-induced withdrawal response, dermal photoreceptors, and their input to the higher-order interneuron RPeD11 in the pond snail *Lymnaea stagnalis*. *J. Exp. Biol.* **213**, 3409-3415.
- Syed, N. I., Bulloch, A. G. M. and Lukowiak, K. (1990). *In vitro* reconstruction of the respiratory central pattern generator of the mollusk *Lymnaea*. *Science* **250**, 282-285.
- Syed, N. I., Ridgway, R. L., Lukowiak, K. and Bulloch, A. G. M. (1992). Transplantation and functional integration of an identified respiratory interneuron in *Lymnaea stagnalis*. *Neuron* **8**, 767-774.
- Taylor, H. H. (1973). The ionic properties of the capsular fluid bathing embryos of *Lymnaea stagnalis* and *Biomphalaria sudanica* (Mollusca: Pulmonata). *J. Exp. Biol.* **59**, 543-564.
- Wedemeyer, H. and Schild, D. (1995). Chemosensitivity of the osphradium of the pond snail *Lymnaea stagnalis*. *J. Exp. Biol.* **198**, 1743-1754.
- Yerkes, R. M. and Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *J. Comp. Neurol. Psychol.* **18**, 459-482.
- Young, J. O. (1975). Preliminary field and laboratory studies on the survival and spawning of several species of Gastropoda in calcium-poor and calcium-rich waters. *J. Mollus. Stud.* **41**, 429-437.