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RESEARCH ARTICLE

Social snails: the effect of social isolation on cognition is dependent on environmental context

Sarah Dalesman* and Ken Lukowiak

Hotchkiss Brain Institute, Department of Physiology and Pharmacology, University of Calgary, HSC 2104, 3330 Hospital Drive NW, Calgary, AB T2N 1P3, Canada

*Author for correspondence (sarah.dalesman@ucalgary.ca)

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SUMMARY

Social isolation is often considered to have negative effects on cognitive function in a wide range of species. Here we assess how environmental context alters the effect of isolation on long-term memory formation (24 h) in the pond snail *Lymnaea stagnalis*. We operantly trained snails to reduce aerial respiration in hypoxia following exposure to one of three social conditions: (1) maintained and trained in groups; (2) maintained in groups, trained in isolation; or (3) maintained and trained in isolation. In addition, snails also experienced four stress exposure levels: control, exposure to low calcium availability, predator kairomone exposure during training or a combination of low calcium and predator kairomones. Snails isolated during training alone demonstrated no difference in memory formation compared with the snails trained in groups. Maintaining snails in social isolation for 8 days prior to training had a neutral effect on memory in control conditions or in the presence of predator kairomones alone. However, social isolation enhanced long-term memory formation in snails exposed to low calcium conditions, a stress that blocks memory formation in snails maintained in groups. Conversely, when exposed to low calcium and predator kairomones combined, grouped snails normally demonstrate long-term memory, but following maintenance in isolation long-term memory was blocked. Therefore, the effect of social isolation on cognitive function is highly dependent on the environmental context in which it is experienced.

Key words: behavioural plasticity, calcium, predator, isolation, learning, memory, *Lymnaea stagnalis*.

INTRODUCTION

Social isolation significantly alters behaviour and physiology in a wide range of animals from invertebrates to mammals. Isolated mammals often exhibit high levels of anxiety that alter other behavioural and physiological traits (Eilam et al., 2011). For example, following periods of isolation, rodents including mice and rats exhibit emotional and cognitive abnormalities (Zhao et al., 2009), changes in open field behaviour (Krohn et al., 2006) and reduced immune function, leading to increased vulnerability to disease proposed to be due to increased stress (Bartolomucci, 2007). Invertebrates also demonstrate responses to social isolation (Sokolowski, 2010), such as retardation of development, reduced formation of neural connections and a decline in behavioural responsiveness in *Caenorhabditis elegans* (Rose et al., 2005). Isolation of *Drosophila melanogaster* Cu/Zn superoxide dismutase mutants reduces individual life span and stress resistance (Ruan and Wu, 2008) and, in wild-type strains of *D. melanogaster*, increases aggression in male–male interactions during the establishment of territories (Hoffmann, 1990). Therefore, the effects of isolation show considerable variation in the behavioural and physiological traits affected.

In addition to other effects, social isolation is often associated with cognitive decline, presumed to be due to stress-associated changes in neurophysiology. There are numerous studies demonstrating effects of isolation on cognition in vertebrates (reviewed in Fone and Porkess, 2008; Cacioppo and Hawkey, 2009); however, the effect in invertebrates is less well studied. Isolating *D. melanogaster* results in a decline in the fibre density in mushroom bodies (Technau, 2007); similarly, isolation of *Apis mellifera ligustica* results in a decline in mushroom body volume (Maleszka et al., 2009). Although learning and memory was not assessed directly in either of these studies, the mushroom bodies play an important role in learning and memory in insects (Fahrbach, 2006). In addition, isolating the sea hare, *Aplysia fasciata*, either during or shortly following training to avoid inedible food blocks long-term memory (LTM) formation (Schwarz et al., 1998).

The great pond snail, *Lymnaea stagnalis*, is commonly used as a model species to study learning and memory because of its easily measurable behaviours and relatively simple nervous system, allowing the neurons controlling those behaviours to be identified (Benjamin et al., 2000; Parvez et al., 2006). LTM formation in this species is malleable, either being enhanced or blocked by a number of different stressors experienced before, during or following learning (Lukowiak et al., 2010). There are already data demonstrating that isolation of individuals leads to significant changes in reproductive behaviour. For example, prolonged isolation of this species during development leads to delayed reproduction and increased size at the onset of reproduction (Koene et al., 2008). *Lymnaea stagnalis* is a primarily out-crossing hermaphrodite (Cain, 1956). Individuals only perform one sexual role at a time during copulation, and although mating is normally reciprocal, isolation of adults for 8 days results in individuals preferentially performing the male role (De Boer et al., 1997). Alteration of copulatory behaviour occurs after snails were isolated using perforated jars maintained within the same aquaria, hence

allowing any waterborne chemicals to freely pass between individuals, but not allowing direct contact. Therefore, perceived isolation, in terms of altering reproductive allocation, may easily occur when snails are at low density in a water body if regular physical contact is not occurring. We were interested in whether this type of social isolation also acts as a stressor that alters LTM formation in *L. stagnalis*, and how social isolation may interact with other environmental stressors to shape memory phenotype, i.e. whether the effects of isolation are context specific.

To test the effect of isolation on LTM formation, snails were either isolated during training alone, which has been shown to block memory in another gastropod, *Aplysia fasciata* (Schwarz et al., 1998), or isolated for 8 days prior to and during training, a period of isolation which is sufficient to alter reproductive behaviour in *L. stagnalis* (Koene and Ter Maat, 2007). We also tested isolation effects in combination with other memory-altering stressors: predator kairomones during training, which enhances LTM (Orr and Lukowiak, 2008; Dalesman et al., 2011c) and exposure to low calcium availability (20 mg l^{-1}), which has previously been found to block LTM (Dalesman et al., 2011b), as well as a combination of these two factors, which are found to interact in grouped snails to produce a memory phenotype identical to controls (Dalesman and Lukowiak, 2011). The effects of each of these additional stressors, low calcium and predator kairomones, on LTM formation are mediated *via* sensing the external environment using a peripheral sensory organ call the osphradium (Il-Han et al., 2010; Dalesman et al., 2011a; Karnik et al., in press), rather than by altering internal homeostasis. *A priori* predictions were that isolated snails would respond to prolonged isolation as a stress that would alter or block LTM formation because of the effects of this period of isolation on reproductive behaviour. However, we also considered that this response may be altered by the presence of other stressors, i.e. low calcium and predator kairomones.

MATERIALS AND METHODS

Adult *Lymnaea stagnalis* (L.), 25±1 mm spire height, were raised from stock obtained from Vrije Universeit in Amsterdam. This population originated from wild snails collected in the 1950s from canals in a polder located near Utrecht. Snails were reared in the Biological Sciences building at the University of Calgary, maintained in artificial pond water (~0.25 g l^{-1} Instant Ocean®, Spectrum Brands Inc., Madison, WI, USA) with the addition of $CaCO_3$ to maintain calcium concentrations >50 mg l^{-1} (Hermann et al., 2009). The animals were fed *ad libitum* with lettuce and Aquamax-carnivorous Grower 600 trout pellets (Purina Mills LLC, St Louis, MO, USA). Snails were transferred into the laboratory 1 week prior to experiments into oxygenated artificial pond water (0.26 g l^{-1} Instant Ocean®) with additional calcium sulphate dehydrate added to make our standard calcium (80 mg l^{-1}) pond water (Dalesman and Lukowiak, 2010). Snails were maintained at room temperature (20±1°C) on a 16 h:8 h light:dark schedule at a stocking density of 1 snail l^{-1} and fed romaine lettuce *ad libitum*.

Training protocol

Lymnaea stagnalis is a bimodal breather, absorbing oxygen directly across its skin in high oxygen conditions; however, in hypoxic conditions it switches to aerial respiration using a basic lung opened to the air *via* the pneumostome (respiratory orifice). Training to reduce aerial respiration in hypoxia was carried out in the same way for all experiments (Lukowiak et al., 1996). Five hundred millilitres of artificial pond water, with either 80 mg l^{-1} or 20 mg l^{-1} [Ca^{2+}] added depending on the treatment protocol, was placed in a 1 l glass beaker. N_2 was then vigorously bubbled through the water for 20 min to make the water hypoxic (≤5% [O_2]). N_2 bubbling was reduced and continued at a low level to maintain hypoxic conditions without disturbing the animals. Snails were then introduced into the beaker in cohorts of six or seven individuals for the grouped training, or individually in both isolation treatments (see below), and allowed to acclimate for 10 min before the start of training. Training was carried out for 30 min, whereby the snail was gently 'poked' on the pneumostome each time it attempted to open it at the water's surface. The snails were then returned to their eumoxic (~100% [O_2]) aquaria for 1 h, after which they received a further 30 min training session identical to the first. Again, snails were returned to eumoxic aquaria following the second training session, and tested for LTM using a protocol identical to the first training session 24 h following training. The number of times the snail attempted to open its pneumostome during both the first (TR1) and second (TR2) training sessions and the testing (test) session were then compared to assess whether the snails had learnt and formed LTM. The snails are considered to have demonstrated learning if the number of attempted openings was significantly lower during TR2 than TR1. If the snail demonstrates LTM, then the number of attempted pneumostome openings during the test session is both significantly lower than during TR1 and not significantly higher than during TR2. Previous work using yoked controls has shown that a reduction in opening attempts during the test phase only occurs when poking is contingent with pneumostome opening, not due to repeated exposure to hypoxia or physical stimulation in a generalised sense (Lukowiak et al., 2003; Lukowiak et al., 2010). Therefore, we are confident that when a snail demonstrates a reduction in pneumostome opening attempts during the test it is due to learning and memory formation. No individual snail was used more than once; *N*-values for each group are provided in the figure legends.

Isolation during training/testing only

We carried out both training and test sessions in individual 1 l beakers (six per training session) in 500 ml of hypoxic water, as outlined above, though in this case each beaker only contained a single individual. Snails were maintained in group conditions in their aquaria at a density of 1 snail l^{-1} between each training session and between the second training session and testing 24 h later.

Maintained and trained in isolation

Snails were placed in individual perforated containers 250 ml in volume within aquaria, whereby waterborne chemicals could travel between animals, but snails were not able to make physical contact (De Boer et al., 1997; Koene and Ter Maat, 2007; Hermann et al., 2009). Water was well aerated to provide eumoxic conditions in each container, and a 2 cm air space was maintained in the top of each isolation chamber to allow snails to carry out aerial respiration whilst isolated. Two aquaria were used for isolation for each treatment group, and aquarium was factored into the initial analysis. Training and testing was carried out in isolation, as outlined above, with the snails maintained in isolated conditions between the training and test sessions.

Handling of the animals in all treatment groups was kept to a minimum. Snails were labelled prior to the 8 day exposure, and then placed into either group or isolation aquaria with sufficient food to last for the duration of the experiment. Subsequently, snails were only handled to move them from the aquaria into the beakers for training and testing. Each individual snail was therefore handled an equal number of times, independent of treatment group.

Low calcium

Acute exposure to a low (20 mg l^{-1}) calcium environment for 1 week prior to and during the training procedure has been found to block the ability of *L. stagnalis* to form LTM (Dalesman et al., 2011b). We exposed snails to either a low (20 mg l^{-1}) or standard (80 mg l^{-1}) calcium environment for 8 days prior to training, either in isolation or in group conditions. Snails were then trained, as outlined above, in the calcium concentration in which they had been held for the previous week.

Predator kairomones

The presence of predator kairomones during training has been found to enhance the ability of *L. stagnalis* to form LTM (Orr and Lukowiak, 2008; Dalesman and Lukowiak, 2011; Dalesman et al., 2011c). Predator kairomone water was produced by placing one crayfish (*Pacifastacus leniusculus*), selected at random from our laboratory population (mantle length 6±1 cm), into 2 l of artificial pond water for 1 h. Pond water was either at our standard calcium concentration (80 mg l^{-1}) or low calcium concentration (20 mg l^{-1}) if combining stressors. Although a low calcium environment may potentially alter the kairomones released by the crayfish, we have found that the enhancing effect on memory formation of crayfish kairomone exposure during training is still present in low calcium conditions when snails are maintained and trained in groups (Dalesman and Lukowiak, 2011). Training in predator kairomones was then carried as outlined above, except that predator kairomone water was used in place of artificial pond water during the two training periods (TR1 and TR2). Testing for memory 24 h later was always carried out in pond water alone.

Statistical analysis

Data were analysed using repeated-measures ANOVA in SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Mauchley's test for sphericity was used to assess homogeneity of variance, and the more conservative Greenhouse–Gesser *P*-values were used where assumptions of homogeneity of variance were not met. Initially, data were analysed for each treatment combination (i.e. social status × stress exposure) separately to assess whether there was any significant effect of cohort (i.e. aquaria in which snails were maintained). As no significance was found, cohorts were grouped for subsequent analysis to allow comparisons among social status groups. Data from different social status groups – (1) control, (2) isolated during training only and (3) isolated prior to and during training – were compared within each treatment group (control, low calcium alone, predator kairomones alone or low calcium combined with predator kairomones). We used training and test period as the within-subject factor (TR1, TR2 and test), and social status (grouped throughout, isolated during training only and isolated prior to and during training) as the between-subject factor in the analyses. When overall significance was identified, *post hoc* pair-wise *t*-tests were used to identify where snails had demonstrated learning and memory, with the *P*-value required for significance corrected to 0.0167 using the Bonferroni correction for multiple tests. *N*-values for each group are given in the figure legends.

RESULTS

Control conditions

In the absence of additional stressors there was a significant overall difference between the training and test periods ($F_{1.63,58.65}$=46.61, P<0.001; Fig. 1). Snails in all social groups demonstrated a significant decline in the number of attempted pneumostome openings between the first (TR1) and second (TR2) training sessions

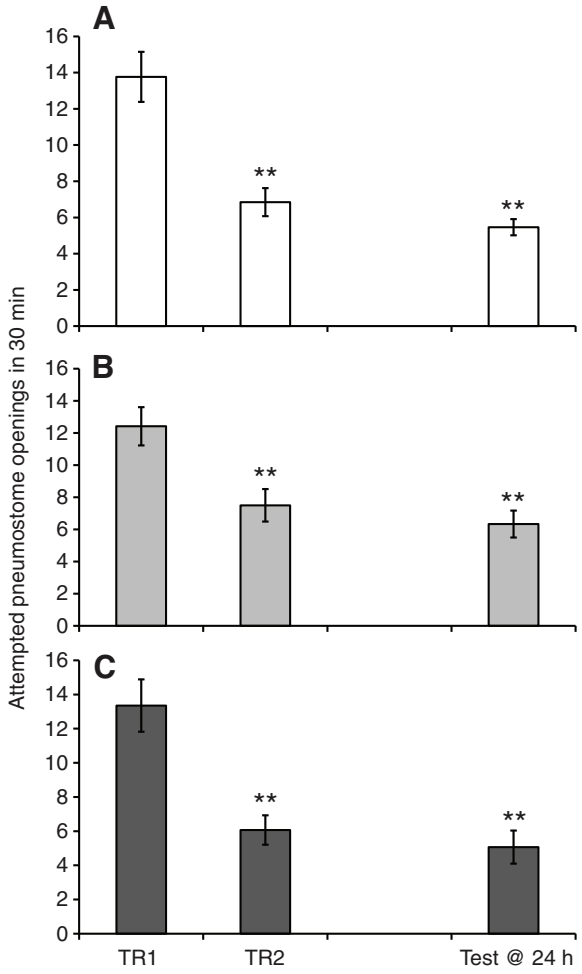

Fig. 1. Memory formation in *Lymnaea stagnalis* under control conditions. Mean ± s.e.m. number of pneumostome opening attempts during training sessions (TR1 and TR2) and test session 24 h later (Test @ 24 h) in control conditions following exposure to different social conditioning: (a) maintained and trained in groups (N=13), (b) maintained in groups and trained in isolation (N=12) and (c) maintained and trained in isolation (N=14). **Significantly different from TR1, P<0.01.

(trained in groups: t=4.56, P=0.001; trained in isolation: t=3.41, P=0.006; trained and maintained in isolation: t=4.06, P=0.001), indicating that learning had occurred, and also a significant decline between TR1 and the memory test (test) at 24 h (trained in groups: t=5.01, P<0.001; trained in isolation: t=3.96, P=0.002; trained and maintained in isolation: t=5.00, P<0.001), also indicating LTM formation in all social groups. There was no significant effect of social grouping, i.e. whether they were trained or maintained in isolation or in a group, on their ability to learn and form memory ($F_{2,36}$=0.26, P=0.77), nor any significant interaction between training and the social status of the snails ($F_{3.26,\ 58.65}$=8.45, P=0.70).

Low calcium exposure

In response to low calcium conditions for 8 days prior to and during training there was a significant interaction effect between the social condition in which snails had been held in and their response to training ($F_{4,74}$=5.548, P=0.001; Fig. 2). Snails that had been maintained in groups and either trained in groups or in isolation demonstrated learning, i.e. a significant decline in the pneumostome opening attempts between TR1 and TR2 (trained in groups: t=5.62,

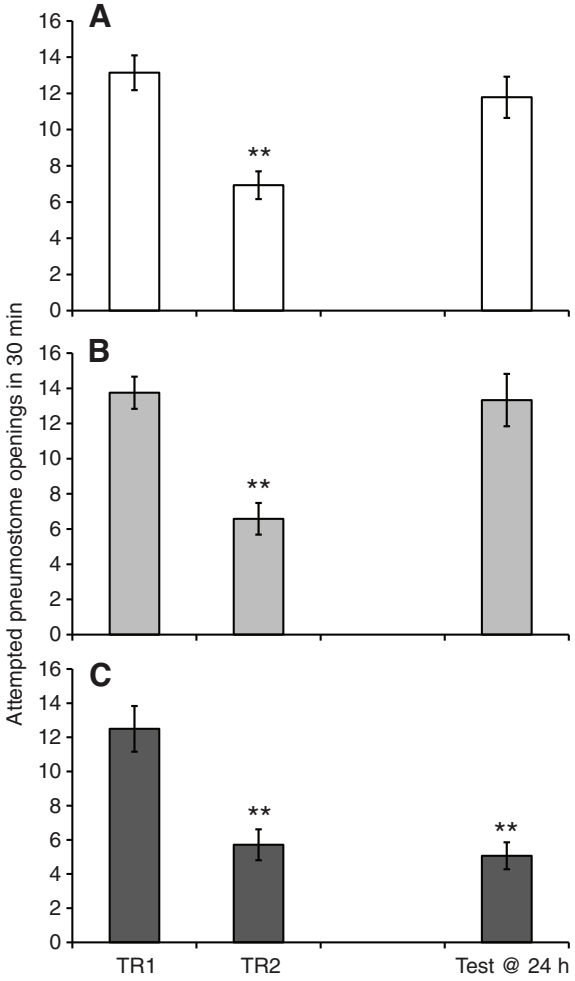

Fig. 2. Memory formation in *L. stagnalis* in low environmental calcium. Mean ± s.e.m. number of pneumostome opening attempts during training sessions (TR1 and TR2) and test session 24 h later (Test @ 24 h) by snails exposed to low environmental calcium (20 mg l^{-1}) prior to and during training/testing, following exposure to different social conditioning: (a) maintained and trained in groups (*N*=14), (b) maintained in groups and trained in isolation (*N*=12) and (c) maintained and trained in isolation (*N*=14). **Significantly different from TR1, *P*<0.01.

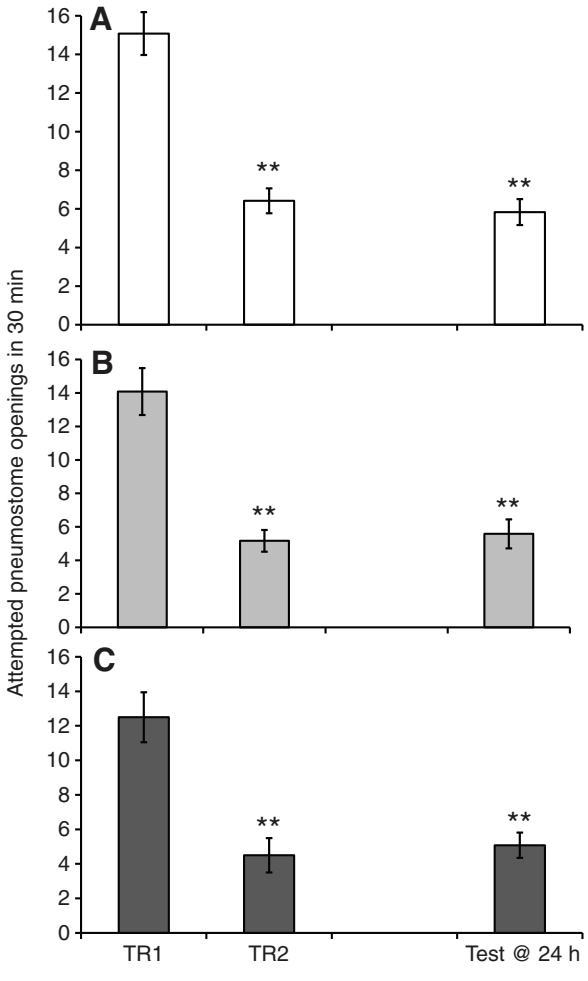

Fig. 3. Memory formation in *L. stagnalis* in the presence of predator kairomones. Mean ± s.e.m. number of pneumostome opening attempts during training sessions (TR1 and TR2) exposed to crayfish kairomones and test session 24 h later (Test @ 24 h) in pond water following exposure to different social conditioning: (a) maintained and trained in groups (*N*=12), (b) maintained in groups and trained in isolation (*N*=12) and (c) maintained and trained in isolation (*N*=12). **Significantly different from TR1, *P*<0.01.

P<0.001; trained in isolation: t=7.07, *P*<0.001); however, they did not show memory 24 h later as there was no difference between TR1 and the test (trained in groups: t=1.10, *P*=0.29; trained in isolation: t=0.32, *P*=0.75). Conversely, snails that had been both maintained and trained in low calcium conditions in isolation demonstrated both learning, i.e. a decline in pneumostome opening attempts between TR1 and TR2 (t=3.95, *P*=0.002), and also memory at 24 h following the second training session, indicated by a significant decline in pneumostome opening attempts between TR1 and the test session (t=4.30, *P*=0.001).

Crayfish kairomones

Following exposure to crayfish kairomones during training there was a significant overall difference between the training and test periods ($F_{1.60,54.55}$=82.12, *P*<0.001; Fig. 3). Snails in all social groups demonstrated a significant decline in the number of attempted pneumostome openings between TR1 and TR2 (trained in groups: t=9.31, *P*<0.001; trained in isolation: t=7.26, *P*<0.001; trained and maintained in isolation: t=4.82, *P*=0.001), indicating that learning had occurred, and also a significant decline between TR1 and the test (trained in groups: t=7.61, *P*<0.001; trained in isolation: t=4.76, *P*=0.001; trained and maintained in isolation: t=4.26, *P*=0.001), also indicating LTM formation in all social groups. There was no significant effect of social grouping, i.e. whether they were trained or maintained in isolation or in a group, on their ability to learn and form memory ($F_{2,34}$=2.24, *P*=0.12), nor any interaction between training and the social status of the snails ($F_{3.21,54.55}$=0.23, *P*=0.89). Therefore, in the presence of predator kairomones, social grouping had no effect on the ability of *L. stagnalis* to learn and form LTM.

Low calcium & crayfish kairomones

Following exposure to low calcium conditions for 8 days and then training in crayfish kairomones, we found a significant interaction effect between the social conditions in which snails had been held in and their response to training ($F_{3.35,56.94}$=5.68, *P*=0.001; Fig. 4). Snails that had been maintained in groups and either trained in groups or in isolation demonstrated learning, i.e. a significant decline in the pneumostome opening attempts between TR1 and TR2

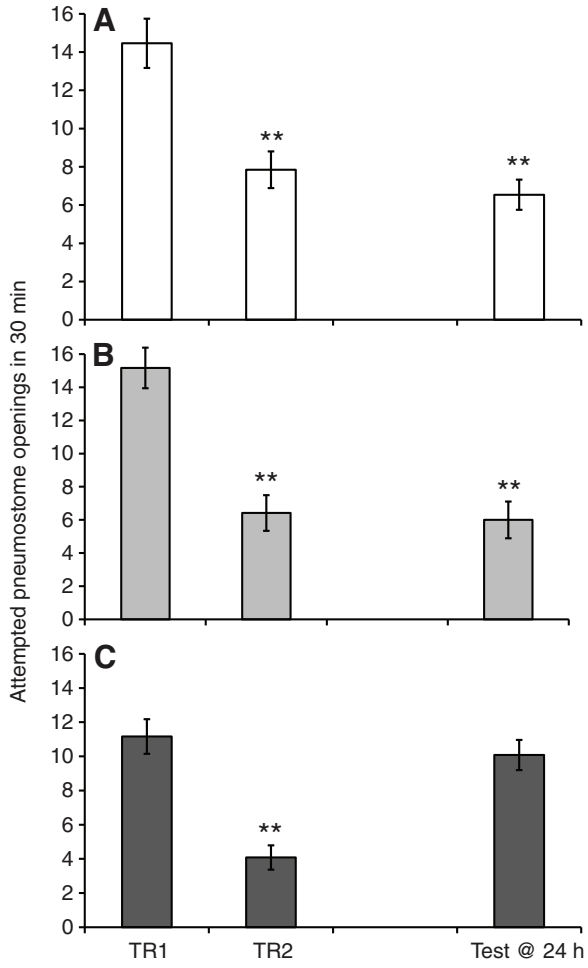

Fig. 4. Memory formation in *L. stagnalis* in low calcium and predator kairomones. Mean ± s.e.m. number of pneumostome opening attempts during training sessions (TR1 and TR2) in crayfish kairomones and test session 24 h later (Test @ 24 h) in pond water by snails exposed to low environmental calcium (20 mg l^{-1}) prior to and during training/testing, following exposure to different social conditioning: (a) maintained and trained in groups (N=14), (b) maintained in groups and trained in isolation (N=12) and (c) maintained and trained in isolation (N=14). **Significantly different from TR1, P<0.01.

(trained in groups: t=3.69, P=0.003; trained in isolation: t=5.08, P<0.001), and also demonstrated memory 24 h later, as there was a significant decline between pneumostome opening attempts during TR1 and the test (trained in groups: t=5.03, P<0.001; trained in isolation: t=4.87, P<0.001). However, snails that were both maintained and trained in isolation, although demonstrating learning, indicated by a significant decline in pneumostome opening attempts between TR1 and TR2 (t=5.66, P<0.001), did not demonstrate LTM 24 h later (TR1 *vs* test: t=0.93, P=0.38).

DISCUSSION

A period of social isolation, sufficient to result in changes in copulatory behaviour (De Boer et al., 1997), is insufficient to alter LTM memory formation in the great pond snail, *L. stagnalis*, when experienced in control conditions. However, when experienced in combination with additional environmental stressors, isolation can significantly affect LTM formation. These effects of social isolation on cognitive function are not consistent, but are highly dependent on the context in which the snail experiences isolation. In low calcium conditions, maintaining snails in social isolation enhances their ability to form LTM, differing from the majority of previous studies that have indicated negative or neutral effects of isolation on cognitive function in a wide range of species (reviewed in Cacioppo and Hawkey, 2009). Conversely, following the addition of predator kairomones during training in low calcium conditions, which was previously found to enhance memory formation (Dalesman and Lukowiak, 2011), isolation blocks LTM formation. In control conditions, or following training in the presence of predator kairomones in a standard calcium environment, there was no effect of isolation on LTM formation. Therefore, the effect that isolation has, either neutral, enhancing or blocking memory formation, is dependent on the presence of other memory-altering stressors.

The enhancing effects of isolation in a low calcium environment may be due to a perceived reduction in competition for scarce resources, or alternatively due to changes in the reproductive behaviour of *L. stagnalis* following isolation. Although there is currently no information about the potential for snails to compete for calcium resources, there is some evidence that calcium requirements may be altered by reproductive activity. Performing primarily as a male during copulation (De Boer et al., 1997; Koene and Ter Maat, 2007) and reducing egg-laying behaviour (Koene and Ter Maat, 2004) following isolation for 8 days will potentially significantly reduce the calcium requirements of an individual snail. Although there is evidence that embryonic snails require external calcium availability for normal growth and development, they are still able to undergo partial development in the absence of environmental calcium (Ebanks et al., 2010), which, alongside [Ca^{2+}] ions being found to be at a greater concentration in recently laid egg capsules compared with ambient conditions (Taylor, 1973), indicates maternal provision of calcium to embryos. Therefore, switching energy investment primarily towards male reproductive output when isolated (De Boer et al., 1997; Koene and Ter Maat, 2007) may significantly reduce calcium requirements of *L. stagnalis*, thereby reducing the stress associated with being held in low calcium conditions.

Although social isolation allowed snails to form LTM in low calcium, when trained in the presence of predator kairomones in low calcium conditions they no longer formed LTM, despite predator kairomones enhancing memory formation in low calcium conditions (Dalesman and Lukowiak, 2011). One explanation may be that the snails are simply experiencing too much stress. The Yerkes-Dodson 'law' suggests that there is an optimum level of stress, below which little attention is paid to training because conditions are already good and an organism has little reason to change; however, above the optimum level conditions are so stressful that the organism is too stressed to pay attention to training (Yerkes and Dodson, 1908; Shors, 2004). This response to stress level has been demonstrated previously in *L. stagnalis*, where too little or too much of a stressful stimulus (KCl bath) experienced prior to training produced a reduction in memory formation (Martens et al., 2007). It seems that this may apply here: where normally predator kairomones would enhance or at the very least have no effect on memory, when experienced alongside two additional environmental stressors, isolation and low calcium availability, it is enough to push our snails 'over the edge', and they are no longer capable of forming LTM. This indicates that at low density in low calcium environments snails may be less able to demonstrate behavioural plasticity, for

example learning about predation risk (Dalesman et al., 2006) or learning to recognise heterospecific alarm cues (Dalesman and Rundle, 2010) when predators are present.

In the absence of any additional stressors (i.e. standard calcium in the absence of predator kairomones) or in the presence of predator kairomones alone, isolation did not alter the ability of snails to form LTM. Potentially, a lack of response to isolation in these conditions is due to waterborne chemicals signalling the presence of conspecifics in the immediate vicinity; therefore, the snails are not aware of their isolation. However, the response to isolation in low calcium conditions (with and without predator kairomones) indicates that this is not the case. The response to crowding in this species, found when individuals are exposed to live snails, but not dead shells or water containing cues from crowded animals, supports the theory that snails are socially aware of direct contact with conspecifics independent of waterborne cues (De Caigny and Lukowiak, 2008). It may be that this period of isolation, in a hermaphrodite that can store sperm from previous mating partners (Koene et al., 2009), is insufficient to alter cognitive function when calcium is not limited.

Isolation during training alone was not sufficient to alter memory formation relative to animals trained in groups, unlike effects seen in *A. fasciata* (Schwarz and Susswein, 1992); therefore, isolation effects were not due to a lack of communication among individuals during the learning procedure. This is in agreement with previous findings, where snails undergoing different training procedures within the same container did not affect the response of other individuals within that container (Sangha et al., 2002), i.e. the difference in LTM formation in snails is not because of a response to training in other individuals. Snails maintained and trained in groups, as well as those maintained in groups but trained in isolation, also respond identically to environmental stress, i.e. LTM was blocked by low environmental calcium availability (Dalesman et al., 2011b) and enhanced by the presence of predator kairomones (Dalesman and Lukowiak, 2011).

The majority of previous studies indicate that isolation has negative or neutral effects on cognitive function in a wide range of species (Cacioppo and Hawkey, 2009), though there are a few exceptions. For example, post-weaning isolated rats may demonstrate enhanced spatial learning in a Morris water maze (Wongwitdecha and Marsden, 1996), though the majority of other studies assessing the effects of isolation on spatial learning found little support for memory enhancement, instead demonstrating either impaired or neutral effects from isolation (reviewed in Fone and Porkess, 2008). Here we found evidence to support negative, neutral and enhanced effects of isolation on memory formation following a single training regime, dependent on the context in which isolation and training were experienced. These data support the idea that inconsistencies in the literature on the effects of isolation on cognition may be due to changes in other environmental variables, for example differences in animal husbandry that are not accounted for in the experimental design. Environmental manipulation of socially isolated animals will further elucidate context-specific effects on cognition as found here.

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