

RESEARCH ARTICLE

Previous motor activity affects the transition from uncertainty to decision making in snails

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ABSTRACT

One of the most widely accepted benefits of enhanced physical activity is an improvement in the symptoms of depression, including the facilitation of decision making. Up until now, these effects have been shown in rodents and humans only. Little is known about their evolutionary origin or biological basis, and the underlying cellular mechanisms also remain relatively elusive. Here, we demonstrate for the first time that preceding motor activity accelerates decision making in an invertebrate, the pond snail *Lymnaea stagnalis*. To investigate decision making in a novel environment, snails, which normally live in water, were placed on a flat dry surface to simulate the potentially threatening consequence of being in an arid environment. This stimulus initiated two distinct phases in snail behaviour: slow circular movements, followed by intense locomotion in a chosen direction. The first phase was prolonged when the test arena was symmetrically lit, compared with one with an apparent gradient of light. However, forced muscular locomotion for 2 h prior to the test promoted the transition from random circular motions to a directional crawl, accompanied by an increase in crawling speed but with no effect on the choice of direction. Intense locomotion for 2 h also produced a strong excitatory effect on the activity of serotonergic neurons in *L. stagnalis*. Our results suggest that the beneficial effects of physical exercise on cognitive performance in mammals might have deep roots in evolution, granting the opportunity to unravel the origins of such effects at the single-neuron and network levels.

KEY WORDS: Locomotion, Arousal, Novelty, Behavioural choice, Behavioural modulation, Antidepressant effects

INTRODUCTION

In rodents and humans, physical activity has been reported to improve memory and learning (van Praag et al., 1999, 2005; Anderson et al., 2000; Cotman et al., 2007; van Praag, 2009; Roig et al., 2012), and to enhance performance in various cognitive tasks such as goal-oriented and effortful behaviours (Chang et al., 2013; Lee et al., 2013; Salmon, 2001; Laurence et al., 2015). One of the most commonly accepted benefits of physical exercise is the reduction in the symptoms of depression, including the facilitation of decision making (Whitton et al., 2015; Salmon, 2001; Greenwood et al., 2003). The cellular and molecular events underlying brain responses to enhanced physical activity have only been partially elucidated. Physical activity has been found to affect neuromodulatory and neurotrophic systems, increasing the levels of serotonin (Kondo and Shimada, 2015),

brain-derived neurotrophic factor (Vaynman et al., 2004), insulin-like growth factor-1 (Carro et al., 2001; Trejo et al., 2001) and vascular endothelial-derived growth factor (Fabel et al., 2003; During and Cao, 2006). These factors in turn stimulate neurogenesis and synaptogenesis, which could account for the increased neurogenesis caused by motor activity in specific brain areas, such as the hippocampus (Lee et al., 2013).

In contrast to the significant amount of data acquired in rodents and humans, surprisingly little is known about whether physical exercise affects brain function in animals belonging to other taxonomic groups. The evolutionary origins and benefits of these effects remain uncharted territory. Presumably, these could appear early in the course of evolution as feedforward stimulating effects of intense locomotion on metabolism and the central nervous system. Functional brain activation could be advantageous for speeding up decision making during fast locomotion in response to rapid changes in the environment. It may also promote behavioural adaptation to the less familiar environments reached after a period of intense locomotion. These presumptions imply that the stimulating effects of intense locomotion on brain function could be widespread throughout the animal kingdom.

Here, we show for the first time that preceding motor activity can affect decision making in a vital situation in an invertebrate, namely the pond snail *Lymnaea stagnalis*. We used a behavioural paradigm in which snails are challenged with the threatening event of being removed from their natural environment (water). When snails are placed on a flat dry surface, they need to decide which way to go in order to reach an aquatic environment. Animals that were forced to use intense muscular crawling for 2 h prior to the experiment chose the escape route significantly faster than those with no preceding motor activity.

Lymnaea stagnalis has been used widely for analysis of the cellular and molecular mechanisms of behaviour (Dyakonova et al., 2015; Koene, 2010; Marra et al., 2013; Pirger et al., 2014; Schmold and Syed, 2012), including studies at the single-cell level (Dyakonova et al., 2009, 2015; Dyakonova and Dyakonova, 2010; Schmold and Syed, 2012). Therefore, our findings also reveal new opportunities for investigating the cellular basis of the intriguing interplay between motor and cognitive activities, which may have deep roots in evolution.

MATERIALS AND METHODS

Animals

Mature specimens of *Lymnaea stagnalis* (Linnaeus 1758) were taken from a breeding colony, kept in dechlorinated tap water at room temperature and fed on lettuce.

A behavioural paradigm to study decision making in a vital situation

The snails, which normally live in water and move by means of cilia, were put into a rectangular arena (60×45 cm) on a flat, dry plastic

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surface, which aimed to simulate the life-threatening event of being out of water (Fig. 1A). In such a situation, snails are expected to choose as promptly as possible a direction of movement to return to an aquatic environment, in order to avoid desiccation.

Two kinds of arena illumination were used: single-side asymmetric illumination (in all experimental series) and central symmetric illumination (in the experimental series performed to compare the impact of environmental asymmetry on decision making). One of the shorter walls of the arena or the ceiling was made of translucent plastic to provide the asymmetric or symmetric illumination, respectively. All other walls were covered with non-translucent matte black film. A 40 W white light bulb placed at 25 cm beside or above the arena served as a source of light. As the arena was not circular, truly symmetric conditions were not achieved even with the light source centred above the arena and the light scattered through the translucent ceiling. Thus, we compared the

apparently asymmetric and more symmetric conditions, calling them asymmetric and symmetric for simplicity.

The light intensity at the centre of the arena (measured 3 cm above the surface with a Proskit MT-4017 luxmeter) was made equal (30 lx) for the two kinds of illumination. In the asymmetric condition, it was 83 and 12 lx near the light and the dark walls, respectively, and in the symmetric condition, it was 23 lx near the left/right borders. No measurable amount of light came to the arena from below. We did not attempt to counteract the temperature gradient (less than 1°C, mini infrared thermometer) caused by the light source, in order to maintain natural conditions, in which more lightened areas are usually warmer.

The movements of each snail were recorded at 15 frames s^{-1} for 15 min with a video camera. The recordings were video tracked using EthoVision XT software (Noldus, The Netherlands) and independently scored manually with RealTimer (OpenScience, Russia). The camera was placed either above (pilot experiments) or under the transparent plastic bottom of the arena. The traces left by the crawling snails were removed with a clean paper towel before each new test.

In the first experimental series, the behaviour of 17 snails in the asymmetric arena illumination was analysed. In the second, double-blind experimental series, the effect of environmental asymmetry on decision making was tested by comparing the behaviour of snails in arenas with asymmetric ($N=8$) and symmetric illumination ($N=12$). Snails (one in each test) were taken randomly from the aquarium, placed on the same central point of the arena in the same orientation (light from the right side in an asymmetric set) and tested in alternating order.

Procedure for investigation of the effects of enhanced motor activity on decision making

In the double-blind experiment, snails were divided into two groups and were maintained individually for 2 h under similar light conditions prior to being placed in the test arena (Fig. 1B). Control group snails ($N=65$) were kept in a cylinder filled with water to a depth of 9 cm so that they could use ciliary locomotion. Snails of the ‘trained’ group ($N=48$) were put into a rectangular tank (25×50 cm) filled with a thin (1 mm) layer of water, which protected the snails from drying but forced them to use crawling (intense muscular locomotion) to compensate for the lack of water supporting the weight of their shells. Individual snails were subsequently placed into the test arena with asymmetric illumination from the trained and control groups in alternating order.

Behavioural analysis

During the analysis, the arena was divided into a circular central zone (diameter 9 cm) and a centred square zone (22×22 cm), which limited the track and scoring analysis (Fig. 1A). The square zone was used to exclude from the analysis snail movements close to the physical borders of the arena, especially near the brightly lit wall. We evaluated (i) the time to the snail’s first turn (change of head orientation greater than 45 deg), (ii) the time to exit from the central zone, and (iii) the time taken to crawl to the virtual border of arena. When a snail did not reach the virtual border, we recorded a value of the total observation time, 15 min. We also recorded the number of turns, the number of head elevations (usually considered orienting behaviour in snails), and the mean period of shell cyclic movements, which is a backward indicator of the frequency of locomotor rhythm. The significance of differences was tested using the Mann–Whitney test. All values are given as the mean with s.e.m. and level of significance.

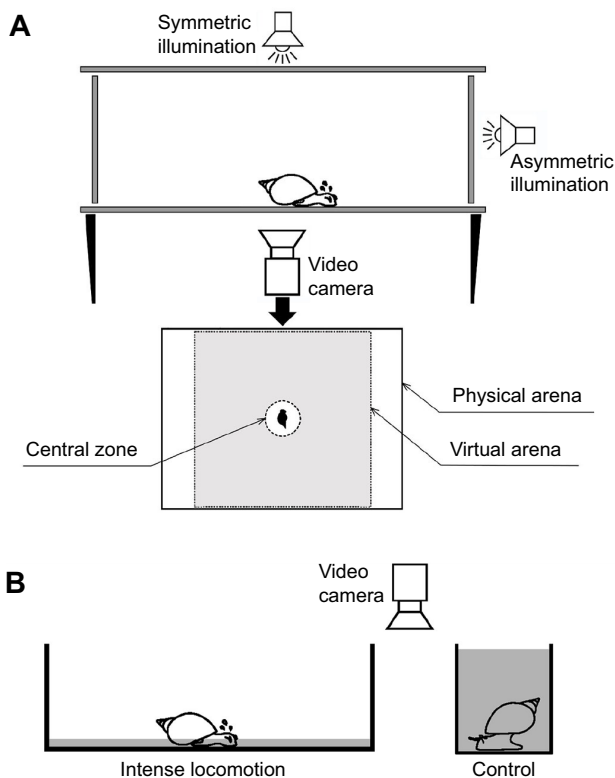


Fig. 1. Schematic representation of the experimental design. (A) Behavioural paradigm for the investigation of decision making in a novel environment. Snails were individually placed on the same central point of a square arena on a flat and dry plastic surface. Two types of illumination were used: one-side asymmetric and central symmetric illumination. All snail movements were tracked and video recorded for 15 min in each experiment by a camera placed either above (pilot experiments) or under the transparent plastic bottom of the arena. For the behavioural analysis, two virtual borders were defined: the central zone border (9 cm around the central point of the arena where the snail was originally placed) and the arena border (22 cm from the central point). (B) Procedure for investigation of the effects of enhanced motor activity. In a double-blind experiment, the snails were divided into two groups: control snails were kept in a cylinder filled with water to a depth of 9 cm so they were able to use ciliary locomotion (no intense locomotion); trained snails were kept in a tank (25×50 cm) filled with a thin (1 mm) layer of water, which protected them from drying but forced them to use crawling (intense muscular locomotion) to compensate for the lack of water supporting the weight of the shell.

RESULTS

Behaviour of snails placed on a dry surface

In the first experimental series, we tested the behaviour of 17 snails randomly taken from the aquarium and placed into the arena with a one-side illumination gradient on a flat and dry plastic surface (Fig. 1A). When placed in the test arena, snails demonstrated two clearly distinct phases in behaviour: (1) uncertain movements such as slow rotations and repeated changes of movement direction, and (2) intense fast locomotion in a chosen direction (Fig. 2). This typical pattern of behaviour was observed in 12 of 17 animals; in 5 snails it was slightly different: either the first phase was reduced to a single turn or additional changes of movement direction were present in the second phase. Most (15 of 17, ~75%) of the snails in the second phase moved towards the light source, and only 2 of 17 made the opposite choice. There were no intermediate choices.

We speculated that a decision-making process may underlie the switch from circular movements to fast locomotion. To further verify this interpretation and the fitness of the behavioural paradigm for studying decision making in snails, we tested whether alterations in the environmental uncertainty and symmetry would affect snail behaviour.

In the symmetric condition, snails started to move in the arena with approximately the same latency as snails tested in the asymmetric condition (Fig. 3A) but spent significantly more time in the central zone (Fig. 3A), demonstrated a significantly higher number of turns (Fig. 3B), and reached the virtual arena border significantly later if at all (Fig. 3A). There were no differences between snails tested in the symmetric and asymmetric conditions in the number of head elevations (Fig. 3B) and in the mean period of shell cyclic movements (Fig. 3C), which is a backward indicator of the frequency of locomotor rhythm. Therefore, the observed differences in the behavioural pattern are likely to be due to the time taken for decision making.

Effects of previous motor activity on the behaviour of snails on a dry surface

We used an asymmetric illumination test to examine the effects of previous motor load. Snails ($N=48$) that were previously forced to use intense locomotion ('trained') demonstrated faster onset of

activity than control animals ($N=65$). The latency of both the first turn (Fig. 4A) and the first shell movement (119.7 ± 8.8 versus 186.8 ± 18.3 s, $P=0.005$, not illustrated) was significantly shorter in the trained group. The first phase also lasted significantly shorter: trained snails made fewer turns (Fig. 4B, Fig. 5), and, as a result, left the central zone faster (Fig. 4A). They also reached the border of the arena earlier (Fig. 4A). The largest difference between trained and control snails was observed in the time spent in the central zone. The mean period of locomotor rhythm was significantly shorter (Fig. 4C, Fig. 5), and the overall speed of locomotion was higher in trained snails. Trained snails also demonstrated a higher number of head elevations (Fig. 4B), usually considered to be an orienting behaviour. The ratio of light/dark choice was the same in the two groups: 75% and 77% of snails moved towards the light in the trained and control groups, respectively. Therefore, it can be said that previous motor activity affected both the time taken to make a decision and the speed of locomotion in a chosen direction; however, it had no effect on the choice of motion direction.

DISCUSSION

The influences of enhanced motor activity on brain function in humans and laboratory mammals have been repeatedly demonstrated and widely discussed in recent decades (Cotman et al., 2007; Chang et al., 2013; Hillman et al., 2008; Kondo and Shimada, 2015; Salmon, 2001; van Praag, 2009). We developed a simple behavioural paradigm for studying risky decision making in a novel environment in an invertebrate animal, the mollusc *L. stagnalis*, a widely used model organism in cellular neurobiology. Using this paradigm, we found that external and internal cues impact behavioural patterns, particularly the delay in decision making. Increased environmental symmetry hampered the choice of direction for intense locomotion in a novel environment, while previous motor activity facilitated decision making.

Risky decision making by snails in a vital situation

We hypothesized that removal of snails from their natural environment could stimulate them to make physical and 'cognitive' efforts to find water and to avoid dehydration. The results of our behavioural experiments agree with this expectation. In nature,

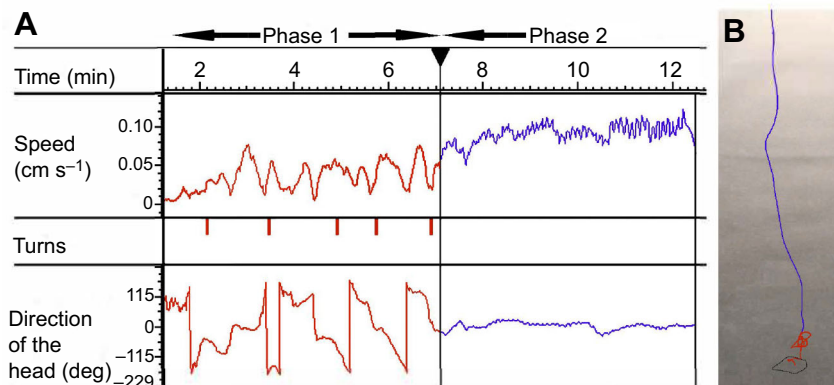


Fig. 2. Parameters of two-phase snail behaviour on a dry surface in an asymmetrically lit arena. (A) Graphical representation of movement speed and turning behaviour for a sample recording. (B) Track of the same recording. The upper horizontal scale shows the time in minutes after the start of the experiment. Speed, measured in cm s^{-1} , was allocated to the time course of the experiment. Each turn indicates a change of the head orientation greater than 45° ; 0° deg is attributed to orientation towards the lit border of the arena. In A and B, two clearly distinct phases can be seen: (1) uncertain movements, characterized by comparatively low speed, stops and repeated changes of movement direction (red) and (2) an intense fast locomotion in a chosen direction, characterizing an increase in the speed of locomotion, and an absence of turns and directional changes (blue). High-frequency oscillations of the speed of movement reflect the rhythmic shell movements that accompany terrestrial muscular crawling in *Lymnaea*. This typical pattern of behaviour was observed in 12 of 17 animals; 15 of 17 snails moved towards the light source, 2 of 17 made the opposite choice.

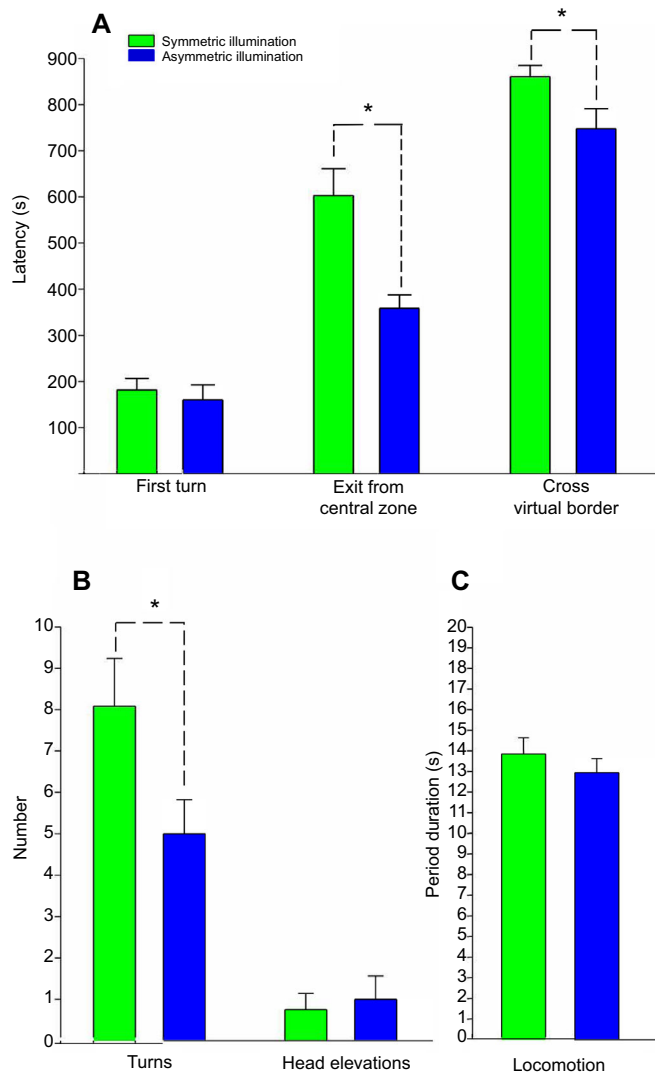


Fig. 3. Effects of type of illumination on the behaviour of snails on a dry surface. (A) Latency of the first turn, exit from the central zone and crossing of the virtual arena border. (B) Number of turns and orienting head elevations during the experiment. (C) Mean period of shell cyclic movements, which is a backward indicator of the frequency of the locomotor rhythm. Green columns indicate snails that were tested in a symmetrically lit arena ($N=12$); blue columns indicate snails that were tested in an asymmetrically lit arena ($N=8$). The significance of differences was tested using the Mann–Whitney test. All values are given as the mean+s.e.m. (* $P<0.05$).

crawling *Lymnaea* snails can sometimes be observed on the shores near water. Therefore, the proposed paradigm is not entirely unnatural for *Lymnaea* ecology and ethology. All tested snails demonstrated activity on the arena's dry surface within the period of observation. Their behaviour contained two distinct phases: uncertain movements and intense fast locomotion in a chosen direction. We interpret this pattern as a transition from uncertainty to decision making: gathering information about the new environment (orienting response), then choosing a direction and, in the second phase, intense 'goal-oriented' locomotion. According to our paradigm, based on the absence of a correct problem solution, a snail invests its time (as a factor of dehydration=drying) and energy in intense locomotion with no guarantee of finding water in the end. It chooses an initial direction on the basis of available environmental cues and, most probably, its behaviour conforms to one of the evolutionarily optimal searching algorithms (Benichou et al., 2011; Salvador et al., 2014).

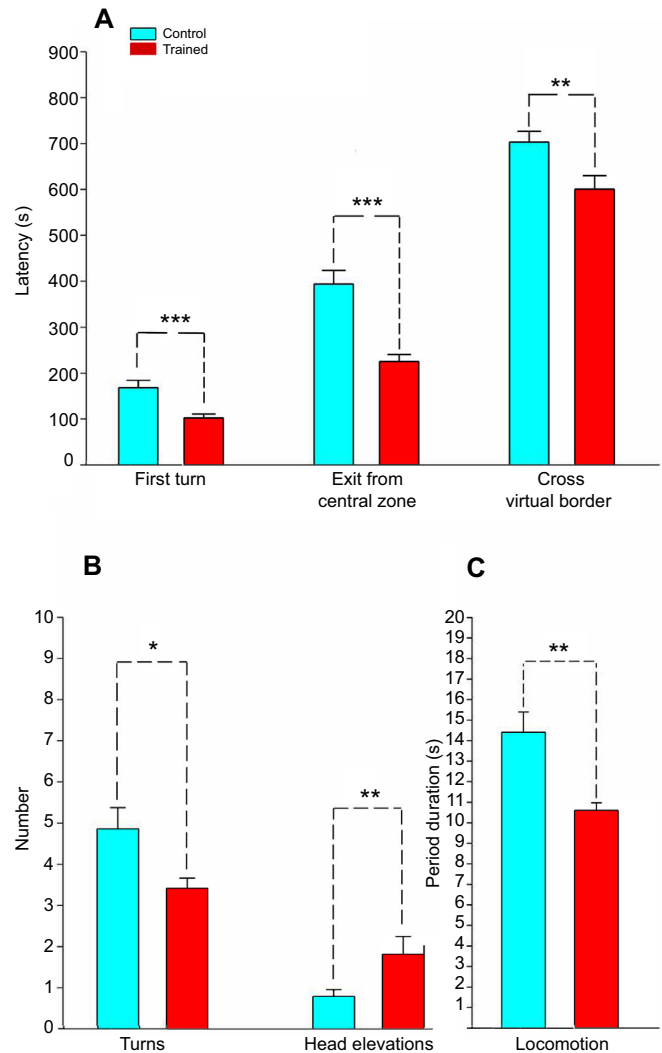


Fig. 4. Effects of previous motor activity on the behaviour of snails on a dry surface. (A) Latency of the first turn, exit from the central zone and crossing of the virtual arena border. (B) Number of turns and orienting head elevations during the experiment. (C) Mean period of shell cyclic movements, which is a backward indicator of the frequency of the locomotor rhythm. Blue columns indicate control snails that were tested in an asymmetrically lit arena ($N=65$); red columns indicate trained snails that were forced to use intense muscular locomotion for 2 h and tested in an asymmetrically lit arena ($N=48$). The significance of differences was tested using the Mann–Whitney test. All values are given as the mean+s.e.m. (* $P<0.05$, ** $P<0.01$ and *** $P<0.001$).

Intermittent target search strategies, which combine phases of slow and fast motion, have been intensively studied, discussed and modelled (Benichou et al., 2011). These strategies have many common features in organisms of different complexity, from unicellular organisms to humans. The mechanisms underlying a biological system's decision to switch from one phase to another are still unknown. Although analysis of the fit of *Lymnaea* behaviour on a dry surface to an intermittent search strategy was not the aim of our investigation, we do not exclude that the switch from uncertain circular movements to fast locomotion may represent an important element of this general strategy.

Most (~75%) of the snails in the second phase moved towards the light source in an asymmetrically lit arena, probably in accordance with the comparatively weak phototaxis known for *Lymnaea* (Vakoliuk and Zhukov, 2000). However, an adaptive sense of

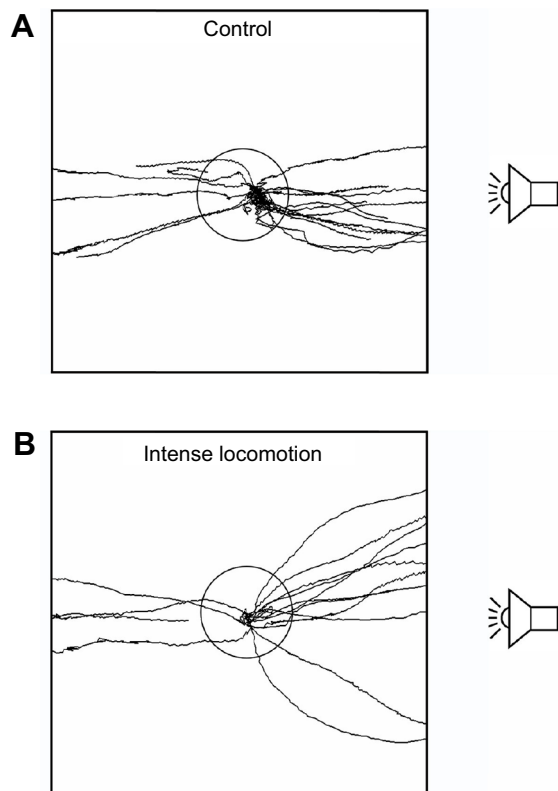


Fig. 5. Overlaid tracks of snails from control and experimental groups tested on the same day. (A) Control snails (no intense locomotion) were tested in an asymmetrically lit arena ($N=18$). (B) Trained snails were forced to use intense muscular locomotion for 2 h and then tested in an asymmetrically lit arena ($N=13$). Tracks presented in B show that trained snails spent less time in the central zone and made fewer turns than control snails (A), although the dark/light choice was not different between A and B.

movement towards light cannot be excluded in the present context, as water surfaces usually reflect light, and have less or no vegetation, which obscures light. Notably, there were no occurrences of an intermediate choice; snails clearly demonstrated preference for light-warm or dark-cool borders. These data show that snails do make a risky behavioural choice in vital circumstances. We use the terms decision-making and behavioural choice in a wider context as elsewhere (Esch et al., 2002; Friesen and Kristan, 2007; Libersat and Gal, 2013), with no speculation on any psychological processes.

Decrease in environmental asymmetry hampers but does not prevent the switch from uncertainty to intense locomotion

Movement strategies are believed to arise from a balance between intrinsic and extrinsic mechanisms (Salvador et al., 2014). We hypothesize that the rotational searching behaviour of *Lymnaea* was initially determined by expectations developed from previous environmental experiences: a recent presence of water suggests a high probability of water remaining, as occurs when snails face dry surfaces and air in their natural ecology. The decision making that marks the switch to the second phase, and, especially, the choice of direction for subsequent fast locomotion, is likely to be performed as information is acquired from the new environment.

Increased uncertainty, or an absence of some external cues that are used for decision-making processes, can significantly hamper decision making (Glimcher et al., 2009; Smith and Ratcliff, 2004;

Stephens and Charnov, 1982). Indeed, in our experiments, the change of arena illumination from asymmetric to symmetric resulted in a significantly delayed behavioural switch from rotation to directional locomotion. The number of orienting turns and duration of the first orienting phase were significantly increased in the symmetrically lit arena test. This finding agrees with the presumption that switching from uncertainty to decision making in *Lymnaea* depends upon available information. Nonetheless, snails made a decision by choosing a direction in the more symmetric environment. This choice is likely to be based on some internal programme that provides a switch of behaviour even when external cues are weak or absent.

Previous motor activity facilitates decision making and promotes effortful behaviour in a vital situation

Previously, we reported some effects of intense locomotion on the behavioural state in *Lymnaea*; namely, a decrease in the defensive responses to shadowing and tactile stimulation and an increase in the speed of aquatic locomotion (D'yakonova, 2014). Here, we used the paradigm of a snail that is forced to make a decision in a vital situation. Snails that were previously forced to use intense locomotion demonstrated a faster onset of movement when put into a new dry arena, made fewer turns, left the central zone faster and reached the arena border earlier, while maintaining the same ratio of light/dark choice preference as control animals. The speed of locomotion was also increased in trained snails. These differences in behaviour cannot be attributed only to a general speeding up of behaviour as the number of turns, and thus the behavioural pattern, was significantly different between the control and trained groups.

We can exclude the effects of novelty-induced stress, because control snails were also kept in a new container with fresh water and more intense light than in the original aquarium. More severe acute stressors, such as occasional damage to a snail or a strong tactile stimulus, caused effects opposite to those of previous motor load, increasing the latency of motor activity in the arena. Nevertheless, we do not exclude the involvement of mild stress effects, which unavoidably accompany intense physical load in both natural and experimental conditions. Some effects of mild stress, such as arousal, and a decrease in the sensitivity to threatening stimuli, resemble the effects of motor load in mammals and also in snails (Heijnen et al., 2016; Kavaliers, 1987).

Facilitation of the decision to make physical effort and increase the speed of locomotion, as observed in our experiments, astonishingly parallels the effects of exercise on willingness to make both physical and cognitive effort in rodents and humans (Eisenberger, 1992; Laurence et al., 2015). These effects are puzzling in terms of cost, because motor load by itself is energetically costly, while both types of activity (mental and physical) are likely to be associated with significant additional costs (for a discussion on the costs of cognitive functions, see Mery and Kawecki, 2003, 2005; Krushinsky, 2015). Proposed explanations of this paradoxical action were attributed to repeated training and presumed a rather complicated subjective estimation of values and intensity of effort in mammals (Eisenberger, 1992; Laurence et al., 2015).

Additionally, in another invertebrate, the cricket *Gryllus bimaculatus*, flying has been reported to restore the ability to fight and win in male crickets (Hofmann and Stevenson, 2000; Stevenson et al., 2005), and to enhance courtship behaviour and mating success (Dyakonova and Krushinsky, 2013). In terms of energy, flying is one of the most expensive forms of motor activity in insects, while fighting, courtship and mating carry high energetic

costs as well. Therefore, evidence now exists for the stimulating effects of motor activity on energy-consuming forms of behaviour in representatives of three major groups of bilaterians: Deutrostomes (humans, rodents), Ecdysozoa (crickets) and Lophotrochozoa (molluscs). If these effects indeed turn out to be widespread, it is likely that they have deep evolutionary roots and a relatively simple explanation in terms of benefits. It has been suggested that feedforward activation of physical and cognitive abilities can be beneficial and even critical for survival in a novel environment reached after a period of intense locomotion.

Perspectives for insight into the molecular and cellular mechanisms of brain modulation by motor activity

The proposed behavioural paradigm makes it possible to perform a wide range of behavioural and pharmacological studies in a simple invertebrate organism with a known cellular background. *Lymnaea stagnalis* snails have been used extensively for the analysis of cellular and molecular mechanisms of locomotion (Syed and Winlow, 1991b; Pavlova, 2010; Longley and Peterman, 2013), feeding (Benjamin and Rose, 1979; Elliott and Benjamin, 1989; Kemenes and Elliott, 1994; Staras et al., 1998, 2003; Alania et al., 2004; Vehovszky et al., 2005; Vavoulis et al., 2007; Chistopolsky and Dyakonova, 2012), respiration (Syed and Winlow, 1991a,b; Tsyganov et al., 2004; Bell et al., 2007), learning and memory (Kemenes et al., 1997, 2002; Kojima et al., 1997; Spencer et al., 1999; Staras et al., 1998; Jones et al., 2003; Sangha et al., 2003; Kemenes et al., 2006; Nikitin et al., 2008; Marra et al., 2013; Mita et al., 2014; Naskar et al., 2014), and decision making (Pirger et al., 2014; Crossley et al., 2016). There are also approaches that have been developed only in this organism, particularly the studies at the single-cell level of freshly isolated, not cultured, neurons (Dyakonova et al., 2009, 2015; Dyakonova and Dyakonova, 2010), and experimental tests of an extracellular chemical microenvironment that has been demonstrated to play a prominent ‘socializing’ role in adjusting single-cell physiology to the network state (Dyakonova et al., 2015). The neurons that control locomotion are at least partially known (Longley and Peterman, 2013). Among them are serotonergic PeA cells forming a big cluster in the pedal ganglia (Syed and Winlow, 1989). As the extra-synaptic release of a neurotransmitter was demonstrated for these neurons, they have been suggested to have a prominent neuromodulatory role (Chistopol’skii and Sakharov, 2003; Dyakonova et al., 2015). Our preliminary results show that 2 h of intense locomotion produce a strong excitatory effect on the activity of PeA cells (68 ± 5 versus 13.5 ± 3 action potential min^{-1} in control, $N=14$, Mann–Whitney U -test, $z=2.96$; $P<0.003$). This effect could be observed for up to 4 h in the isolated central ganglia kept in physiological solution. Serotonin facilitates many forms of behaviour, including learning and memory in molluscs (Gillette, 2006). The role of serotonin in exercise-induced brain plasticity is evidenced in mammals (Kondo and Shimada, 2015). Therefore, the possible involvement of serotonergic mechanisms in the effects of intense locomotion in *Lymnaea* is interesting in terms of the evolutionarily conserved neurochemical basis of these effects.

Acknowledgements

We thank Dr M. Yu. Stepanichev for providing us with the opportunity to analyse our video recordings with the Noldus EthoVision software. We thank Prof. D. A. Sakharov and Prof. F. Libersat for critical reading of this manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

T.A.K.: performed research and analyzed data. D.D.V.: designed research and analyzed data. V.E.D.: designed research, analyzed data and wrote the paper.

Funding

This work was supported by Russian Foundation for Basic Research (RFBR) grants 14-04-00537 and 14-04-00875.

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