

## Light-dark decision making in snails: Do preceding light conditions matter?

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### ABSTRACT

We have recently demonstrated that preceding motor activity can facilitate decision-making not only in humans and mammals but also in molluscs.<sup>5</sup> In the behavioral paradigm used, snails *Lymnaea stagnalis* were removed from their natural environment (water) and placed in a dry, asymmetrically lit arena from which they had to decide which way to go in order to reach an aquatic environment.

One possible explanation of the observed effects of preceding motor activity was that it could affect the snail's memory of light conditions that corresponded to its previous aquatic habitat. Here we report experimental results discarding this hypothesis. We suggest that preceding intense locomotion is likely to facilitate decision-making by increasing the level of confidence.

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The influence of exercise on brain function in humans and mammals has been repeatedly demonstrated in the past decades.<sup>1-4</sup> Recently, we suggested that these effects may have deep roots in evolution, and experimentally demonstrated that preceding motor activity facilitates decision-making in an invertebrate animal, the mollusc *Lymnaea stagnalis*.<sup>5</sup> We used a behavioral paradigm in which these predominantly aquatic snails were challenged with the threatening event of being removed from water by putting them in a dry, asymmetrically lit arena. According to this paradigm, a snail invests its time (as a factor of dehydration) and energy into intense locomotion (crawling) with no guarantee of finding water in the end. It chooses an initial direction on the basis of available environmental cues and, most probably, performs one of the evolutionarily optimal searching algorithms.<sup>6</sup> The snails' behavioral pattern consisted of two distinct phases: first, slow circular movements and second, intense crawling locomotion in a chosen direction. We found that forced intense locomotion for two hours prior to the test promoted a faster transition from circular motions to a directional crawl, accompanied by an increase in the crawling speed but with no effect on the choice of direction. Most of the snails (~75%) moved toward the light source in the second phase. There were neither intermediate choices in the control nor in the experimental group. The importance of light asymmetry for snail decision-making was further confirmed in experiments with symmetrically lit arena. The first phase

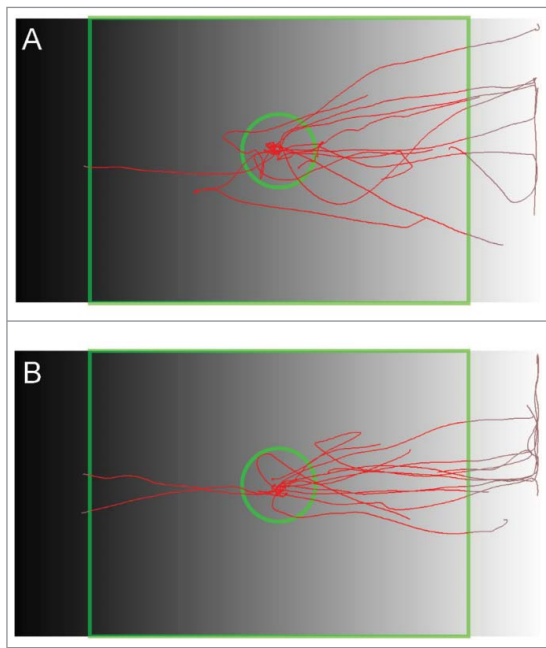
of behavior was prolonged and decision-making was delayed compared to the tests with an apparent gradient of light.<sup>5</sup>

In the experiments published,<sup>5</sup> the light conditions were equalized for two hours prior to the test in control and exercising snails, and during this interval the light intensity was higher compared to the test arena in any of its parts. The hypothesis we propose and test here states that the snail associates the light conditions preceding the test with the presence of water, while the intense locomotion affects this association or the memory recall. We report the results of experiments aimed at elucidating this issue.

We kept snails in aquatic containers in different light conditions prior to the test performed in the dry arena. The first container was lit brighter (90 lux) than the area near the bright wall of the experimental arena (83 lux) and the second one was lit darker (8 lux) than the area near the dark wall of the arena (12 lux).

First, we tested the effect of light-dark preconditioning for 2 hours which corresponds to the duration of motor load, or forced intense locomotion, in our previous experiments.<sup>5</sup> Only one of eight snails chose the dark wall to crawl to, and this snail was light-preconditioned.

After a longer preconditioning protocol (24 hours), 2 of 20 snails crawled to the dark wall, one from the dark-preconditioned group (Fig. 1A, note, that in the figures, the data from 2 or 24-hours preconditioned snails are

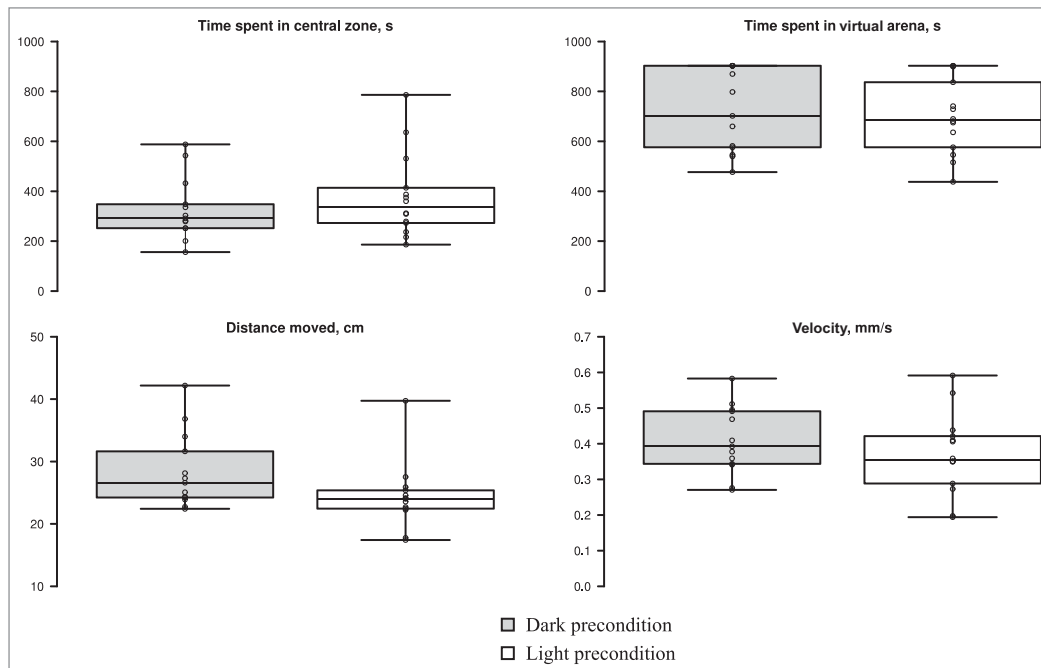


**Figure 1.** Overlaid tracks of snails from dark- and light-reconditioned groups tested on the same day. Data from 2- and 24-hour preconditioned snails are merged. Central zone and virtual arena zone are shown in green. Track statistics were analyzed only within the virtual arena boundaries. A: Dark-preconditioned snails (water container was lit darker (8 lux) than the area near the dark wall of the arena),  $n = 13$ . B: Light-preconditioned snails (water container was lit brighter (90 lux) than the area near the bright wall of the test arena),  $n = 14$ .

merged) and one from the light-preconditioned group (Fig. 1B). We found no significant difference in any other parameters of behavior (the time spent in the central zone and in the virtual arena, distance moved, velocity) between the two groups (Fig. 2).

Therefore neither 2 nor 24 hours of light-dark preconditioning affected light-dark choice of snails in the arena. Snails preferred the bright wall of the arena irrespective of previous light conditions, while the number of snails which had chosen to crawl to the darker part of the arena remained within the previously reported level of 25%. The known phototaxis behavior in *Lymnaea*<sup>7</sup> seems also to be unaffected by the internal state (preceding motor load).<sup>5</sup> Our results contrast with recently reported findings on another invertebrate, the fruit fly *Drosophila melanogaster*,<sup>8</sup> where phototaxis was controlled by internal cues. Thus, we conclude that light conditions immediately preceding the test can be discarded as a key factor influencing the decision-making process of snails in our experiments.

Our next question would be whether motor load facilitates decision-making by increasing the level of confidence. Confidence is typically considered as a complex metacognitive process based on statistical computations and performed by prefrontal cortex in humans.<sup>9-11</sup> However, confidence is also known to depend upon the general behavioral state of the organism, homeostasis,



**Figure 2.** Parameters of snails behavior in asymmetrically lit dry arena after light-dark preconditioning: time spent in the central zone (s), time spent in the virtual arena (s), distance moved (cm), velocity (mm/s). Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend to minimum and maximum values; data points are plotted as open circles.  $n = 13$  (dark-preconditioned, gray),  $n = 14$  (light-preconditioned).

emotion and feeling.<sup>12,13</sup> We speculate that an internal regulation of confidence is adaptive even for simple organisms, since it allows certain flexibility in the trade-off between speed and accuracy of decision-making. Recently, confidence and its underlying neurophysiological mechanisms have been studied in rodents.<sup>10</sup> To elucidate the neurochemical and cellular basis of confidence in an invertebrate organism would be a challenging task for the future. *Lymnaea* is a promising model in this respect, as it is a well-known organism in cellular neurobiology and allows to perform electrophysiological and molecular studies at the single cell level.<sup>14-20</sup>

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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