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THE INFLUENCE OF LIGHT ON LOCOMOTION IN THE GASTROPOD *MELIBE LEONINA*

JAMES M. NEWCOMB[a,b,*], KADDEE A. LAWRENCE[a,b,†] and WINSOR H. WATSON III[a,b]

[a]*Zoology Department & Center for Marine Biology, University of New Hampshire Durham, NH, 03824, USA;* [b]*Friday Harbor Laboratory, University of Washington, Friday Harbor, WA, 98250, USA*

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In this study, we investigated the effects of light on both the locomotion of intact animals and the swim motor program expressed by isolated brains in the gastropod *Melibe leonina*. Spontaneous locomotion (crawling and swimming) was examined during a period of natural lighting (L:D) to establish normal behavior, and then under two different light regimes: constant darkness (D:D) and constant light (L:L). In L:D, there was significantly more locomotor activity at night than during the day and this pattern continued in D:D. However, in L:L, activity was substantially reduced at all times. Using isolated brain preparations, we further demonstrated that the swim motor program was rapidly inhibited by light, and that this inhibition was mediated by the eyes. These results indicate that *M. leonina* displays a nocturnal activity pattern, and that light has a strong inhibitory effect on locomotion in the intact animal and on the swim motor program expressed by the isolated brain.

Keywords: Central pattern generator; Circadian rhythm; Crawling; Nocturnal; Nudibranch; Swimming

INTRODUCTION

Light is an important exogenous cue involved in resetting circadian clocks and modulating activity patterns in a wide array of organisms, including fungi, plants and animals. This modulation of activity can conform to annual, seasonal, lunar or daily rhythms of light cues. The predictability of exogenous light rhythms enables many animals to synchronize their activity with that of other organisms in their environment, including both prey and predators. It is not uncommon for many organisms to have a nocturnal pattern of locomotor activity to minimize competition for resources and avoid potential predators (Cloudsley-Thompson, 1961).

The influence of light on the locomotor activity of gastropods has been investigated in a number of species including *Aplysia californica* (Kupfermann, 1968; Kupfermann and Carew, 1974), *Arion ater* (Lewis, 1969), *Bulla gouldiana* (Block and Davenport, 1982),

*Corresponding author. Present address: Department of Biology, Georgia State University, PO Box 4010, Atlanta, GA 30302-4010, USA. Tel.: 404-651-0920. Fax: 404-651-2509. E-mail: jnewcomb@gsu.edu
†Present Address: Highline Community College, Des Moines, WA, 98198-9800, USA

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Bursatella leachi (Block and Roberts, 1981; Ramos *et al.*, 1995), *Helisoma trivolvis* (Kavaliers, 1981), *Limax maximus* (Sokolove *et al.*, 1977), and *Melanoides tuberculata* (Beeston and Morgan, 1979). These animals display three different patterns of activity: some are nocturnal (*B. gouldiana*, *H. trivolvis*, and *L. maximus*), some diurnal (*A. californica*, *A. ater*, *B. leachi*), and one is even distinctly crepuscular (*M. tuberculata*). However, while the influence of light on locomotor activity has been extensively studied at the behavioral level, very few studies have attempted to determine how light influences the neural circuits that control locomotion. This is probably due, in large part, to the fact that these circuits are either unknown or not clearly defined for most of the aforementioned species. In contrast, while some of the neural circuits controlling locomotion have been identified in other gastropods, such as *Aplysia brasiliana* (von der Porten *et al.*, 1980; Gamkrelidze *et al.*, 1995), *Clione limacina* (Arshavsky *et al.*, 1985), *Melibe leonina* (Watson *et al.*, 2001, 2002), *Pleurobranchaea californica* (Jing and Gillette, 1995, 1999), and *Tritonia diomedea* (Getting *et al.*, 1980; Lennard *et al.*, 1980; Frost and Katz, 1996), little is known about the effects of light on locomotor activity patterns in these animals. The goal of this study was to investigate the influence of light on locomotion in *M. leonina*, both at the behavioral and neurophysiological levels.

We chose to work with the marine nudibranch *M. leonina* because it offers several advantages over other model systems for investigating the neural mechanisms underlying the interaction of light with a defined locomotor central pattern generator (CPG). First, there is already some evidence suggesting that light affects locomotion in *M. leonina* (Hurst, 1968; Ajeska and Nybakken, 1976; Watson *et al.*, 2001). Second, the CPG controlling swimming in this animal is well understood and is comprised of only four neurons (Watson *et al.*, 2001) that operate as a classic half-center oscillator to produce the alternating lateral bending movements of swimming (Lawrence and Watson, 2002; see http://zoology.unh.edu/faculty/win/win%20homepage%20links/research%20page/Melibe/melibeswimming.htm for videos of *M. leonina* swimming). Third, the eyes are located directly on the brain, providing a convenient opportunity to directly examine the effects of light on the swim CPG in isolated brains. Thus, *M. leonina* appears to be especially fitting for investigating how light influences a locomotor CPG.

In this study, we found that intact *M. leonina* tended to crawl further, and swam more frequently, at night and they maintained this pattern of nocturnal activity in constant darkness. However, in constant light, locomotion was severely reduced at all times. *M. leonina* also had a tendency to swim more in response to noxious stimuli during the night than during the day. Electrophysiological experiments with isolated brains demonstrated that light inhibits the swim motor program, and that this inhibition is mediated by the eyes. The results of these experiments suggest that light is an important factor influencing locomotion in *M. leonina*, and provides a rare example of light-induced modulation of a locomotor CPG.

METHODS

Animal Collection and Maintenance

Adult *M. leonina* (5–10 cm in length) were collected during the winter months from subtidal eelgrass beds near Friday Harbor Laboratories (FHL) on San Juan Island,

WA by the authors and David Duggins. Experiments were carried out at FHL, where animals were kept in flow-through seawater tables at ambient temperatures (~10°C), and at the University of New Hampshire, where they were maintained in recirculating seawater tanks at 10°C. All *M. leonina* were kept on a light/dark regime (9 h of light and 15 h of darkness [9L:15D]) that approximated the natural light cycle of their habitat during the winter months.

Locomotor Activity Experiments

Locomotor activity levels of *M. leonina* were examined under a natural light cycle (15D:9L) followed by one of two different lighting regimes: constant darkness (D:D) or constant light (L:L). Around noon, animals were placed in individual 2-L containers containing 1 L of seawater, and placed inside a light-tight enclosure in a cold room (10°C). Monitoring of activity did not begin until 4 pm to give the animals a chance to adjust to their new surroundings. Each container was circular and had a piece of PVC pipe (3.8 cm diameter) in the middle, cut to be the same height as the container and with holes to allow for water and gas exchange. Aeration was accomplished by bubbling water inside the pipe, so as not to affect water flow in the rest of the container. The location of the pipe at the center of the circular container created a "racetrack" shape, facilitating measurement of locomotion as animals moved around the track. The containers were also translucent, and a pie chart with alternating black and white sections was placed underneath the container to provide contrast for viewing the animals from above and serve as a means of measuring the distance that animals moved. Daytime lighting was provided by a 30-W incandescent light bulb placed above the animals (~200 lux). An infrared light array (<0.01 lux, Fuhrman Diversified, Inc., Seabrook, TX) was used to allow observation in darkness with an infrared-sensitive camera (CVC-320WP Waterproof CCD Camera, Rock House Products, Middletown, NY). These luminance values are comparable to the light that *M. leonina* experiences in their natural habitat, where they are exposed to illuminance ranging from 0.01 lux or less at night, to 40–630 lux during the day (as measured with a LI-COR LI-185 submersible light meter [Lincoln, NE]). After approximately 4 h of acclimation in the test chambers in the light, all experimental trials began when the lights were turned off at 4 pm. Animals were exposed to 15 h of darkness (considered "night") immediately followed by 9 h of light (considered "day") ($n = 19$), after which the lighting regime was switched to D:D ($n = 10$) or L:L ($n = 9$) for another 24–39 h (Fig. 1).

Crawling and swimming activity for all experiments was monitored with an infrared camera and videotaped (Panasonic AG-RT600P time-lapse VCR) for later analysis. Videotapes were manually analysed by counting the number of spontaneous swim episodes per hour and measuring the distance that animals crawled in relation to the pie chart located underneath the containers. *M. leonina* is typically active, and animals that continually crawled less than 1 cm/h or did not swim at all under normal light conditions were considered unhealthy and were not included in the results. There were large variations in the activity levels between animals which required normalization of the data. This was accomplished for each animal by noting the maximum distance crawled in an hour for each individual and then dividing the distance crawled each hour by this maximum hourly distance. This was then converted to a percentage by multiplying by 100, thereby providing an hourly "percentage of maximum

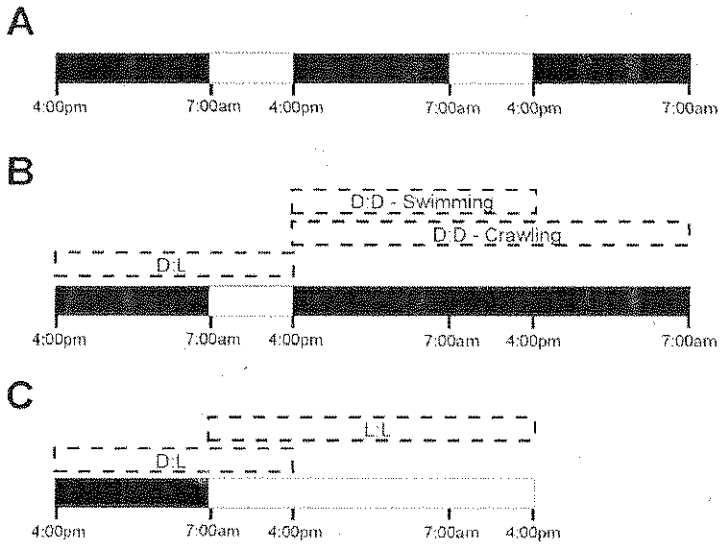

FIGURE 1 Schematic diagram representing experimental design of the behavioral activity experiments. (A) Representation of normal 15D:9L light cycle that animals were housed under previous to experiments. Black boxes represent periods of darkness and white boxes represent periods of light. (B) Ten animals were initially kept under normal L:D conditions for 24 h and then switched to constant darkness (D:D) for an additional 24–39 h. Boxes outlined with dotted lines represent the periods of time used for analysing crawling and swimming activity in L:D and D:D (see Results text for further explanation). (C) Nine animals were also initially kept under normal L:D conditions and then switched to constant light (L:L) for an additional 24 h.

activity" for each animal. Data are presented as averages ± standard error and statistical comparisons of activity levels were done with repeated measures ANOVAs and Tukey *post-hoc* tests unless otherwise noted.

Influence of Light on Stimulus-induced Swimming

The most effective stimulus for eliciting swimming in *M. leonina* is the touch of a tube foot from the predatory seastar *Pycnopodia helianthoides*. Therefore, in natural light cycles (15D:9L), animals ($n = 64$) were tested for their likelihood to swim in response to a touch with a single seastar tube foot during the night (10 pm) and the day (10 am). Since we needed to see the animals in the "dark" to apply the tube foot, a 40-W red ceramic-coated bulb (~5 lux, light wavelength > 600 nm) was used for night-time illumination instead of the infrared lights used in the aforementioned locomotor activity experiments. Preliminary experiments indicated that light wavelengths above 600 nm had no influence on the swim motor program in isolated brains with intact eyes. Daytime illumination was provided by a 60-W incandescent light bulb (~800 lux). Individual *M. leonina* were placed into 8-L plastic aquaria with flow-through seawater and left alone for 12 h to adjust to their new surroundings. After application of the tube foot at the indicated times, it was noted whether or not individual animals swam.

Influence of Light on the Swim Motor Program Expressed in Isolated Brains

Animals were anesthetized by chilling and their brains (left and right cerebropleural and pedal ganglia) were removed by cutting all nerve roots except for the pedal–pedal connectives that travel around the ventral surface of the esophagus. Isolated brains were pinned in a Sylgard-lined, 2 mL recording dish embedded in a hollow aluminum platform. Coolant or ambient seawater was circulated through the aluminum platform to keep the recording chamber at 8–12°C. Intracellular recordings were obtained using 20–50 MΩ microelectrodes filled with 2 M potassium acetate. Intracellular amplifiers (A-M Systems, Inc., Neuroprobe Amplifier Model 1600, Sequim, WA and Dagan 8700 Cell Explorer, Minneapolis, MN) were used to monitor neuron activity and their output was viewed on an oscilloscope and recorded on a chart recorder (Astro-Med, Inc. Dash IV, West Warwick, RI).

In the first series of experiments, dark-adapted brains that were spontaneously expressing the swim motor program ($n = 26$) were exposed to bright light from a fiber optic illuminator to determine if light inhibited swimming. Dark adaptation was accomplished by leaving the brain in total darkness (< 0.01 lux) for 30 min. To control for the possibility that brains were responding to any change in illumination and not just an increase in light levels, the same preparations ($n = 26$) were also examined for a response to a rapid decrease in light after light adaptation (30 min in bright light [> 1000 lux]). In the second series of experiments, brains ($n = 6$) were used to determine the light threshold necessary to inhibit swimming. The room lights were turned off after impaling swim interneurons or putative swim motorneurons (Watson *et al.*, 2001, 2002) and the preparation was left in total darkness for 30 min (< 0.01 lux). The preparation was then exposed to varying light intensities using a rheostat-controlled fiber optic illuminator located 6 ft from the preparation. The amount of light actually impinging on the brain was measured with a HOBO light meter (Onset, Bourne MA) located next to the recording chamber. The brains were exposed to a measured light intensity for approximately 30 s and the intensity was changed every 5 min. Threshold was determined to be the least amount of light that was able to cause complete cessation of fictive swimming. Threshold was examined again in these same brains ($n = 6$) after excising both eyes, which are connected to the brain by a very short ocular nerve and are located between the cerebropleural and pedal ganglia (see Fig. 1A in Newcomb and Watson, 2001). The electrodes were removed from swim motorneurons during surgery and then cells were re-penetrated. Threshold tests were repeated as described above.

RESULTS

Locomotor Activity Experiments

In the L:D/D:D experiments, all 10 animals exhibited robust nocturnal crawling in the initial L:D phase. However, 3 of the 10 animals were kept in constant darkness for only 24 h instead of 36 h. To enable paired statistical analysis which included a "third night", these 3 animals were therefore excluded from the following analysis of crawling activity. The remaining 7 animals crawled significantly more at night than during the day under the normal light conditions (15D:9L) at the beginning of the experiment (Fig. 2). The average distance crawled per hour during the night was $20.6 \pm 4.2\%$ of maximum, which was significantly farther than during the day

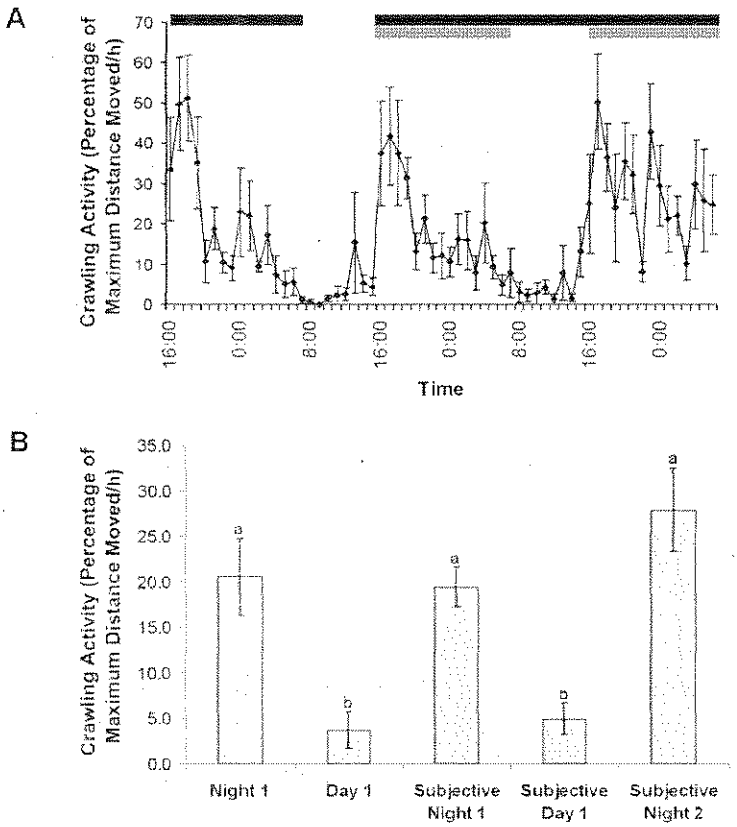

FIGURE 2 Crawling activity in a natural light regime (15D:9L) followed by constant darkness (D:D). (A) Average, normalized crawling activity for 7 animals. Due to large variation between animals, the data were normalized by converting distance crawled per hour to a percentage of maximum hourly distance traveled for each animal (see Methods for further explanation). The black bars indicate darkness and the gray bars indicate subjective night during D:D. Nocturnal activity is clearly evident and this pattern continued in 39 h of D:D. The highest amount of activity under both lighting regimes was in the first half of each night. (B) Average crawling activity for each night and day ($n = 7$). Crawling activity was significantly higher during the night than in the daytime and this pattern continued in 39 h of constant darkness. Bars with different letters are significantly different from each other ($p < 0.05$). Error bars represent standard error.

($3.7 \pm 2.0\%$, Fig. 2B). Animals were placed in their containers at noon and displayed little if any movement during the subsequent 4 h, until it was dark (data not shown), suggesting that exploration of their new surroundings did not commence until nightfall.

In constant darkness, a nocturnal pattern of activity continued. Crawling activity during the subjective daytime (hours that would have been daytime in a normal L:D cycle) was $4.9 \pm 1.7\%$ of maximum, which was significantly lower than both the first subjective night ($19.5 \pm 2.2\%$) and the second subjective night ($27.9 \pm 4.6\%$, Fig. 2B). The distance traveled during the subjective day and nights in D:D was very similar to the distance moved by the same animals during respective periods before

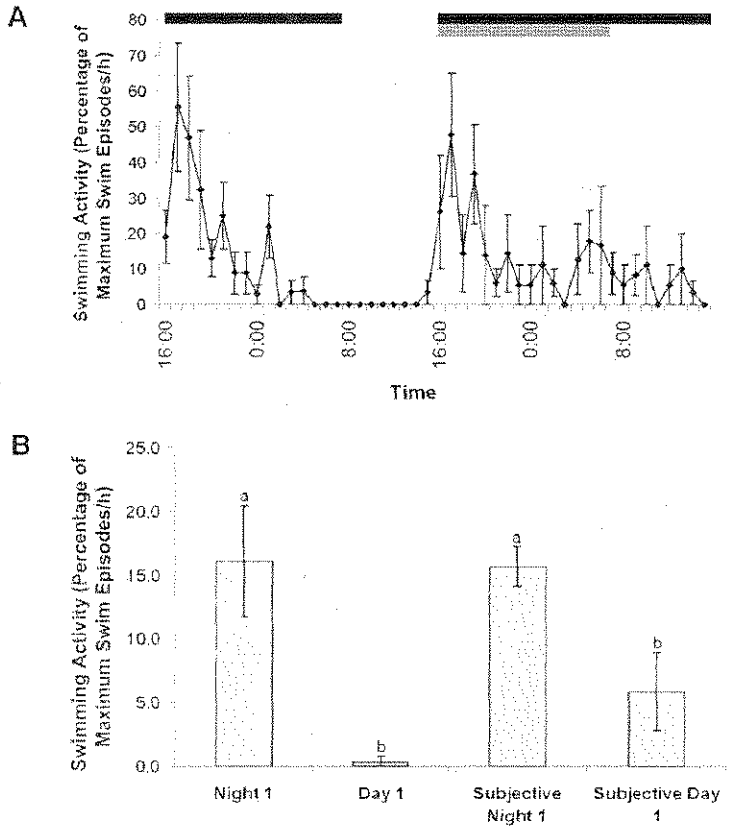

FIGURE 3 Swimming activity in a natural light regime (15D:9L) followed by constant darkness (D:D). (A) Average, normalized swimming activity for 6 animals. The black bars indicate darkness and the gray bars indicate subjective night during D:D. As with crawling (Fig. 2), swimming activity followed a nocturnal pattern, which continued in D:D. Swimming occurred primarily in the first half of each night and was almost nonexistent when the light was on (Day 1). (B) Swimming activity was significantly higher at night than during the day, even during the subjective night in D:D (bars with different letters are significantly different from each other, $p < 0.05$).

entering D:D ($p > 0.05$, Fig. 2), providing further evidence that putative endogenous activity patterns remained unchanged in D:D.

In addition to crawling, *M. leonina* also spontaneously swims. Four of the 10 animals tested in the L:D/D:D experiment never swam, regardless of the phase of the light cycle. Swimming data from the remaining 6 animals were analysed only through subjective day 1 (Fig. 1B) to increase our sample size and incorporate the three animals mentioned above which were only exposed to 24 h of constant darkness. *M. leonina* displayed a nocturnal activity pattern of swimming that resembled that of crawling (Fig. 3). The average number of swim episodes per hour during the first night ($16.1 \pm 4.3\%$ of maximum) was significantly higher than during the day ($0.4 \pm 0.4\%$, Fig. 3B). This pattern continued through 24 h of constant darkness with significantly more swim episodes during the subjective night ($15.7 \pm 1.6\%$ of maximum) than subjective day ($5.9 \pm 3.0\%$, Fig. 3B). These data indicate that *M. leonina* has a nocturnal pattern of swimming, as well as crawling.

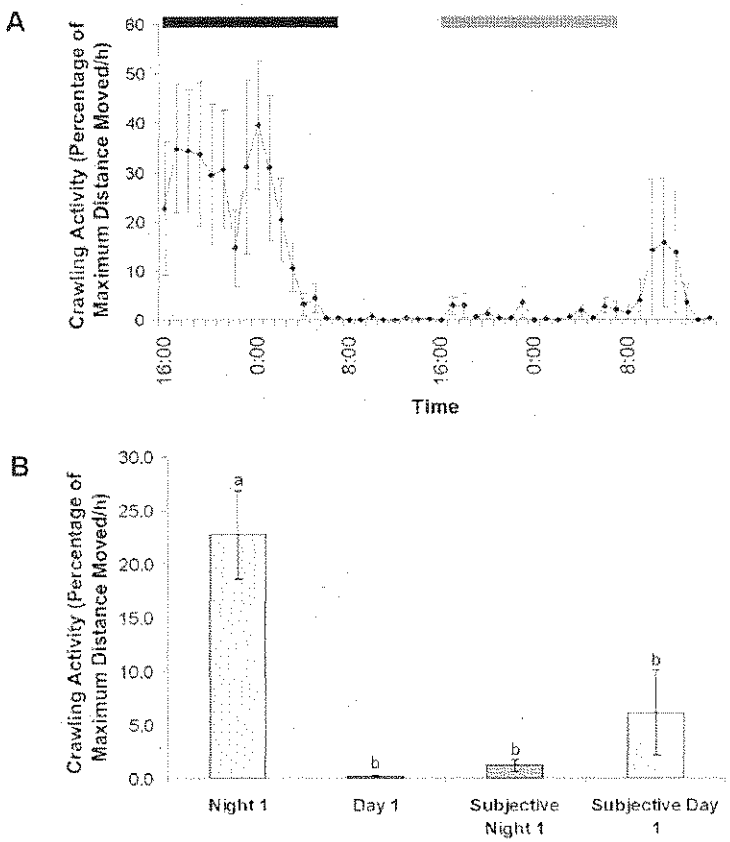

FIGURE 4 Crawling activity in a natural light regime (15D:9L) followed by constant light (L:L). (A) Average, normalized crawling activity for 7 animals. The black bar indicates darkness and the gray bar indicates subjective night during L:L. Animals exhibited a nocturnal pattern of activity during the initial L:D lighting regime, as in Figs. 2 and 3. However, this pattern did not continue under L:L. (B) Swimming activity was virtually nonexistent in the light, with the exception of a small, insignificant burst of activity during subjective day 1. Bars with different letters are significantly different from each other ($p < 0.01$).

Locomotor patterns were also monitored in L:D/L:L. In these experiments, two of the 9 animals displayed very little activity (< 1 cm/h average crawling activity and no swim episodes), even in the initial L:D phase of the experiment, and were therefore excluded from the following analyses. For the remaining 7 animals, the distance crawled during the first night ($22.7 \pm 4.2\%$ of maximum) was once again significantly greater than activity during the day ($0.2 \pm 0.1\%$, Fig. 4), mimicking the nocturnal activity pattern observed during the initial L:D phase of the D:D experiments. However, in constant light, crawling activity remained low during both subjective night ($1.2 \pm 0.5\%$ of maximum) and subjective day ($6.1 \pm 1.0\%$, Fig. 4B). Thus, light appeared to suppress any putative endogenous driver of activity that may normally have caused an increase in activity during the night.

Melibe leonina exhibited a nocturnal pattern of swim activity during the initial L:D phase of the experiment, as in the D:D experiments (Figs. 3 and 5). The average number of swim episodes during the night ($16.8 \pm 3.1\%$ of maximum) was significantly higher than during the day ($0.0 \pm 0.0\%$, Fig. 5B). However, as with crawling, swimming

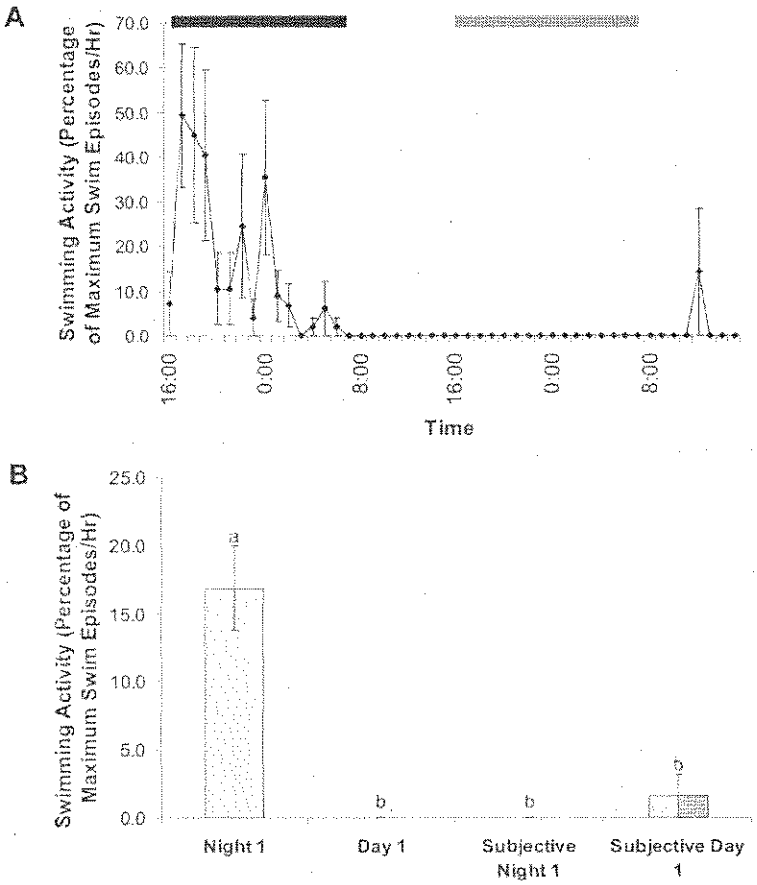

FIGURE 5 Swimming activity in a natural light regime (15D:9L) followed by constant light (L:L). (A) Average, normalized swimming activity for the same 7 animals as in Fig. 4. The black bar indicates darkness and the gray bar indicates subjective night during L:L. Swimming activity followed the same pattern as crawling activity under the same light conditions (Fig. 4) with an initial nocturnal pattern of activity suppressed in L:L. (B) There was no significant difference in swimming activity once the light was on. Bars with different letters are significantly different from each other ($p < 0.001$).

was continuously suppressed in constant light, with swim activity of $0.0 \pm 0.0\%$ and $1.6 \pm 1.6\%$ for subjective night and day respectively (Fig. 5B).

Influence of Light on Stimulus-induced Swimming

We then investigated whether stimulus-induced swimming was also more prevalent at night than during the day. Under natural lighting conditions (15D:9L), significantly more animals swam in response to a *Pycnopodia helianthoides* tube foot during the night (64%) than during the day (42%) ($p < 0.05$, Fisher's exact test, $n = 64$). These results support the aforementioned data concerning spontaneous bouts of locomotion and suggest that light reduces the probability that *M. leonina* will spontaneously swim or swim in response to a noxious stimulus.

Influence of Light on the Swim Motor Program Expressed in Isolated Brains

We next investigated whether light influenced the neural circuit controlling swimming in *M. leonina*. In low light or dark conditions, swim interneurons and putative swim motorneurons in isolated brains typically exhibit continuous bursting, indicative of a swim motor program. This fictive swimming was rapidly inhibited when brains were illuminated and swimming resumed when the light was shut off (Fig. 6A). This effect was repeatable over multiple preparations (18 of 26 brains tested).

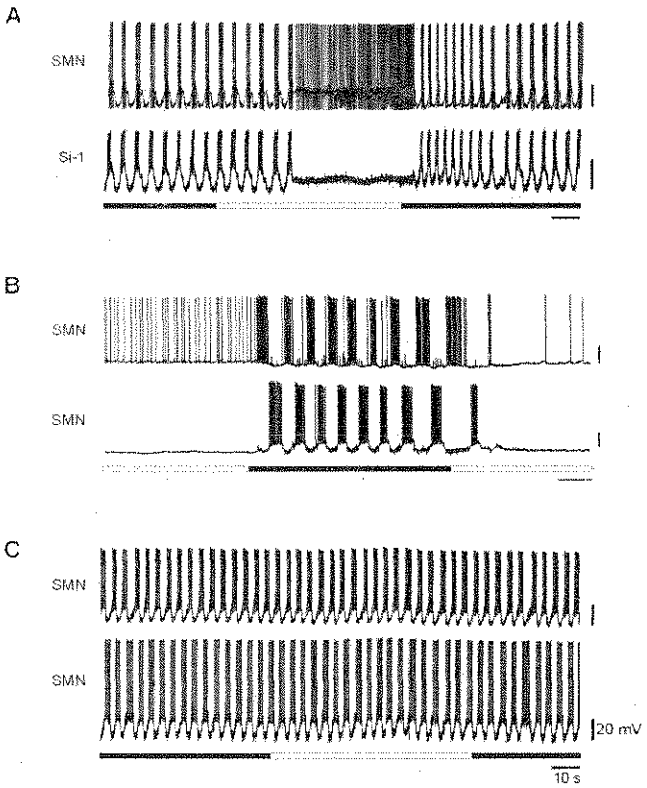

FIGURE 6 Light alters fictive swimming. (A) Intracellular recordings from a putative swim motorneuron (SMN, top trace) and the ipsilateral swim interneuron 1 (Si-1, bottom trace) on the right side of the brain. Swim neurons in the isolated brain typically display a continuous bursting pattern in darkness or dim lighting (black bars). In this example, the onset of light from a fiber optic lamp (white bar) after dark-adaptation (>30 min in darkness) resulted in inhibition of the swim motor program. This effect was reversible, with rhythmic bursting reappearing after the offset of light. The putative swim motorneuron fired irregularly during the inhibition of swimming, presumably due to input from swim interneuron 2 which can be irregularly active when not bursting (personal observation). (B) Offset of light has an opposite effect to onset. In a light-adapted preparation (>30 min in light) contralateral swim motorneurons were firing irregularly (top trace) or were quiescent (bottom trace). Offset of light (black bar) caused a rapid initiation of the swim motor pattern which was reversibly inhibited upon onset of the light after ~1 min. (C) *M. leonina*'s eyes, which are located directly on the brain, mediate the light-induced inhibition of the swim motor program. After removal of the eyes from the same brain used in "A", intracellular recordings from a different set of swim neurons show that onset of light (white bar) in a dark-adapted preparation had no effect on the swim motor pattern. Though it was often difficult to get recordings from the same neurons before and after removal of the eyes, it should be noted that when the eyes were present, supra-threshold light consistently elicited disruption of rhythmic bursting in every swim neuron examined, and that this disruption was never seen after removal of the eyes ($n = 6$).

The latency between illumination and disruption of the firing pattern was approximately 4–8 s for preparations that were dark adapted and 10–15 s in preparations that were not dark-adapted. In control preparations that were light-adapted, the swim motor pattern was either quiescent or irregular. A sudden offset of light in these preparations either elicited a swim motor pattern or stabilized an ongoing pattern (Fig. 6B). The fact that diametric visual stimuli induced opposite results suggests that the light-induced inhibition of the swim motor pattern is not merely a startle response.

The minimum amount of light necessary to inhibit the swim motor program (threshold) in dark-adapted brains was 0.7 ± 0.2 lux, which is roughly equivalent to the amount of light ~1.3 m from a candle flame. This level of light was sufficient to completely disrupt the swim motor program in all 6 preparations tested for threshold. In some preparations, light intensities slightly below threshold could cause an increase in the periodicity of the swim motor program (data not shown). In all animals tested, light stimuli above threshold did not affect the swim motor program in the same brains after ablation of the eyes, suggesting that light-induced inhibition of swimming is mediated by the eyes (Fig. 6C).

DISCUSSION

The findings from this study suggest that *M. leonina* are nocturnal and that light has an inhibitory effect on both crawling and swimming. Experiments carried out in total darkness indicate that *M. leonina* may have an endogenous circadian rhythm, though it is still unclear whether this rhythm is robust enough to last longer than 39 h, or whether it can be entrained by light. Light not only inhibits general locomotor activity, but it also reduces the likelihood of swimming in response to a noxious stimulus. This inhibition is also manifested in isolated brains and this inhibition of fictive swimming is mediated by the eyes. Therefore, our results demonstrate that light is an important factor influencing two types of locomotion in *M. leonina*.

Previous accounts of *M. leonina* activity patterns in the field were based on anecdotal data and were inconclusive. Hurst (1968) observed that juvenile *M. leonina* swam to a light that was placed in the water at night, indicating a positive phototaxis. However, this observation may also indicate nocturnal swimming activity. In one of the first papers about *M. leonina*, Agersborg (1921) noted that *M. leonina* primarily feeds at night, and in 50 h of SCUBA field observations, Ajeska and Nybakken (1976) occasionally saw *M. leonina* swimming, but never during daylight hours. Our more recent observations, both in the laboratory and the field, are consistent with these reports as well. Thus, based on previous observations and the data provided in this report, we conclude that *M. leonina* typically displays a nocturnal pattern of activity.

Experiments with isolated brains indicated that the threshold for light-induced inhibition of the swim motor program was 0.7 ± 0.2 lux. Measurements of light levels in their natural habitat in shallow eelgrass beds around San Juan Island in the Puget Sound indicate that *M. leonina* is typically exposed to light levels ranging from 0.01 lux or less at night, to 40–630 lux during the day, depending on the time of the day and weather conditions. Even though the eyes of *M. leonina* are located

directly on the brain, the integument is transparent. Therefore 40–630 lux is probably more than enough light to cause inhibition of swimming during the daytime, while night-time illumination (< 0.01 lux) is dim enough to release the swim network from light-induced inhibition. Our behavioral and electrophysiological experiments involved abrupt changes of light, which do not exactly mimic the gradual light changes that *M. leonina* experiences in its natural habitat. Therefore, these experiments are not directly comparable to activity in the field and merely suggest likely activity patterns and a potential neural mechanism.

The propensity for *M. leonina* to move around more at night, as opposed to during the daytime, may be an attempt to avoid diurnal predators. However, the only reports in the literature of predation on *M. leonina* are by a few crab species (Mauzey *et al.*, 1968; Ajeska and Nybakken, 1976; Bickell-Page, 1991) and the seastar *Crossaster papposus* (Mauzey *et al.*, 1968; Bickell-Page, 1991). Though the seastar *Pycnopodia helianthoides* has never been seen eating a *M. leonina*, it should be noted that the touch of a single tube foot from this predator can elicit swimming in *M. leonina*, as indicated in this study. In addition to swimming, which may be used at times as an escape behavior, *M. leonina* also has glands that release a potentially repugnant chemical that may deter predators (Bickell-Page, 1991; Barsby *et al.*, 2002) and it is known to autotomize its cerata in response to aversive stimuli (Bickell-Page, 1989). Due to limited observations of *M. leonina* in the field, it is still possible that there are unidentified predators of *M. leonina* that make night-time activity more beneficial than daytime activity.

Melibe leonina is commonly found in eelgrass (*Zostera marina*) and kelp (*Macrocystis integrifolia*) beds (Hurst, 1968; Ajeska and Nybakken, 1976; Gosliner, 1987), which means that they typically traverse a vertical, as opposed to benthic, habitat. Therefore, it may be that light influences daily fluctuations in their vertical distribution, resulting in diel vertical movement. Diel vertical movement on macroalgae has been demonstrated in two other mollusks, *Aplysia parvula* and *Phasianotrochus eximius* (Rogers *et al.*, 1998). Both of these organisms occupied significantly higher positions on their host algae at night compared to during the day. While adult *M. leonina* often feeds on free-swimming crustaceans, such as gammarids, caprellids and copepods (Hurst, 1968; Watson and Trimarchi, 1992; Watson and Chester, 1993), it also consumes epifaunal crustaceans, bivalve spat and epiphytic algae directly from the eelgrass and kelp blades (Ajeska and Nybakken, 1976; personal observation). Epiphytes can be more abundant in the canopy of macrophytes (Brawley, 1992), and thus would be more attractive to a nocturnally foraging *M. leonina*. It is therefore possible that *M. leonina* may exhibit nocturnal activity to maximize foraging, while minimizing predation (Zaret and Suffern, 1976; Stich and Lampert, 1981; Bollens and Frost, 1989). Preliminary laboratory experiments have not supported diel vertical movement in *M. leonina* (personal observation), but it is difficult to accurately mimic the location of prey and predators in the water column, in the lab. More rigorous investigation may elucidate whether *M. leonina* undergoes diel vertical movement and whether this type of movement is influenced by light.

While crawling is the primary form of locomotion in gastropods, many species, including *M. leonina*, also swim. In some species, such as *Tritonia diomedea* (Willows *et al.*, 1973) and *Pleurobranchaea californica* (Jing and Gillette, 1999), swimming is typically used only as an escape response from predators. Other gastropods, such as

Clione limacina, are pelagic and swim constantly (Satterlie *et al.*, 1985). Only a few gastropods, such as the sea hare *Aplysia brasiliana* and *M. leonina*, will spontaneously swim, though the behavioral reasons for this are currently unclear. *Aplysia brasiliana* swims via parapodial flapping (McPherson and Blankenship, 1991; Gamkrelidze *et al.*, 1995) and it is thought that it may swim more commonly at night (Gamkrelidze *et al.*, 1995), which is similar to activity patterns of *M. leonina*, as indicated in this study.

In the current study, the continuation of the activity rhythm in constant darkness suggests that there may be an endogenous component to locomotion in *M. leonina*, though longer-term experiments will be necessary to confirm this. Many other gastropods are known to have an endogenous activity rhythm, including *Aplysia californica* (Jacklet, 1969; Lickey *et al.*, 1977), *Arion ater* (Lewis, 1969), *Bulla gouldiana* (Block and Davenport, 1982), *Bursatella leachi* (Block and Roberts, 1981), *Helisoma trivolvis* (Kavaliers, 1981), *Limax maximus* (Beiswanger *et al.*, 1981), and *Melanoides tuberculata* (Beeston and Morgan, 1979), though, of these species, only *B. gouldiana*, *H. trivolvis* and *L. maximus* are nocturnal. The continued activity rhythm seen in constant darkness with *M. leonina* disappeared in constant light. In addition, light had a strong inhibitory effect on activity in natural light conditions, reduced swimming in response to a noxious stimulus, and inhibited the swim motor program in isolated brains. Thus, it appears that the inhibitory effect of light on locomotion in *M. leonina* may be stronger than any putative endogenous component of the activity rhythm. These results are also consistent with Aschoff's (1960) circadian rule that constant light causes an overall decrease of activity in nocturnal animals. Of the three nocturnal species listed above, only *H. trivolvis* has been examined under constant light conditions (Kavaliers, 1981). In that case, circadian rhythms persisted in the presence of constant light. However, this has not been investigated in *B. gouldiana* or *L. maximus*, so it is difficult to determine whether these strong inhibitory effects of light seen in *M. leonina* are common or unique among other nocturnal gastropod species.

The rapid light-induced inhibition of the swim motor program in isolated *M. leonina* brains (Fig. 6A) could be interpreted as a startle response. Modulation of locomotion by rapid changes in light exposure have been previously documented, especially in regards to shadow responses (Stoll *et al.*, 1976; Meyer *et al.*, 1981; Young and Chia, 1985; Buskey *et al.*, 1986; Easter and Nicola, 1996; Forward *et al.*, 1996; Campbell and Nash, 1998; Leys *et al.*, 2002), though some animals do exhibit locomotor changes induced by transient increases of light as well (Saladin, 1982; Buskey and Swift, 1983; Koehler *et al.*, 1987; Batty, 1989; Grober, 1990; Otis and Gilly, 1990). It does not appear that the changes in expression of the *M. leonina* swim motor pattern elicited by light are the result of a type of startle response for two main reasons. First, increases and decreases in light levels result in opposite changes in fictive swimming, which is unlikely if both stimuli are triggering a startle response. Second, our unpublished observations of the responses of intact animals to changes in illumination suggest that light mostly serves to modulate neural circuits, rather than actually eliciting strong behavioral effects. For example, we rarely observe animals start to swim when the lights are turned off or stop swimming when the lights are turned on. Furthermore, *M. leonina* are often observed swimming and crawling in the daytime, even though our behavioral and neurophysiological data indicate that light changes the probability that they will express these behaviors.

Our results from isolated brain preparations suggest that the eyes mediate the light-induced inhibition of the swim motor program in *M. leonina*, since removal of the eyes abolishes this inhibition. While this may seem straightforward, it should be noted that extraocular photoreception has been well documented in a number of gastropods (*Aplysia californica*: Block *et al.*, 1974; *Aplysia fasciata*: Stoll, 1979; *Helisoma trivolvis*: Kavaliers, 1981; *Helix pomatia*: Pasic *et al.*, 1977; *Hermissenda crassicornis*: Jerussi and Alkon, 1981; *Limax maximus*: Beiswanger *et al.*, 1981; *Lymnaea stagnalis*: Chono *et al.*, 2002; and *Onchidium verruculatum*: Hisano *et al.*, 1972), and even plays a role in circadian rhythms and activity patterns in *Aplysia californica*, *H. trivolvis* and *L. maximus*. While our results do not rule out a potential for peripheral extraocular photoreceptors (e.g. in peripheral ganglia or skin) to affect *M. leonina* locomotor activity, they do suggest that any such receptors located in the central nervous system are not influential in short-term, light-induced inhibition of swimming, at least at the light levels that we used.

Behavioral studies have previously examined the effects of light on locomotion in a wide array of species, but less is known about how light affects neural networks that control locomotion. The neural effects of light via the pineal eye have been examined in swimming of *Xenopus laevis* tadpoles (Jamieson and Roberts, 2000). These tadpoles exhibit increased pineal activity and upward swimming in response to light dimming, since they tend to attach to the underside of floating objects that cast shadows. Ventral root recordings indicated an increased rate of fictive swimming in immobilized tadpoles in response to light dimming. In crayfish (*Procambarus clarkii*), illumination of the last abdominal ganglion initiates the pattern generating network for backward walking by exciting caudal photoreceptors (Simon and Edwards, 1990). However, while there is some understanding of the locomotor circuits in *X. laevis* (Roberts *et al.*, 1998) and *P. clarkii* (Kovac, 1974a,b; Moore and Larimer, 1987), the swim circuit in *M. leonina* is much simpler and it is much easier to record from individual CPG neurons to directly monitor neuronal responses to exogenous cues. Therefore, *M. leonina* may be a more useful preparation to investigate how light influences locomotor CPGs.

Previous work with *M. leonina* has indicated that the gaseous neurotransmitter nitric oxide (NO) can inhibit swimming in both semi-intact preparations and in isolated brains (Newcomb and Watson, 2002), and this effect is very similar to the light-induced inhibition seen in the current study. There are only two nitrergic neurons in *M. leonina*, located near each optic ganglion, and these cells have projections in the neuropil near the optic ganglia and the pedal ganglia (Newcomb and Watson, 2001), where part of the swim CPG, as well as putative swim motorneurons, reside (Watson *et al.*, 2002). Further studies are needed to determine if light-induced inhibition of swimming is mediated by NO.

In conclusion, our results indicate that the gastropod *M. leonina* displays a nocturnal pattern of locomotor activity. Light inhibits crawling and both spontaneous and escape swimming in intact animals. Light also disrupts the swim motor pattern in isolated brains. Due to their relatively simple swim CPG, and the location of the eyes directly on the brain, we have been able to demonstrate light-induced changes in a locomotor CPG. This lays the groundwork for potentially investigating neural mechanisms underlying the effects of light on a simple, defined locomotor neural network.

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