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Two Components of Nocturnal Locomotor Suppression by Light

Lawrence P. Morin[1,2], **Pablo J. Lituma**[1], and **Keith M. Studholme**[1]

[1] Department of Psychiatry, Stony Brook University Medical Center, Stony Brook, NY 11794

[2] Program in Neuroscience, Stony Brook University Medical Center, Stony Brook, NY 11794

Abstract

In nocturnal rodents, millisecond light ("flash") stimuli can induce both a large circadian rhythm phase shift and an associated state change from highly active to quiescence followed by behavioral sleep. Suppression of locomotion ("negative masking") is an easily measured correlate of the state change. The present mouse studies used both flashes and longer light stimuli ("pulses") to distinguish initiation from maintenance effects of light on locomotor suppression and to determine whether the locomotor suppression exhibits temporal integration as is thought to be characteristic of phase shift responses to pulse, but not flash, stimuli. In Expt. 1, locomotor suppression increased with irradiance (0.01–100 $\mu W/cm^2$), in accordance with previous reports. It also increased with stimulus duration (3–3000 sec), but interpretation of this result is complicated by the ability of light to both initiate and maintain locomotor suppression. In Expt. 2, an irradiance response curve was determined using a stimulus series of 10 flashes, 2 msec each, with total flash energy varying from 0.0025 – 110.0 J/m^2. This included a test for temporal integration in which the effects of two equal energy series of flashes were compared, but which differed in the number of flashes per series (10 vs 100). The 10 flash series more effectively elicited locomotor suppression than the 100 flash series, a result consistent with prior observations involving flash-induced phase shifts. In Expt. 3, exposure of mice to an 11 hr light stimulus yielded irradiance-dependent locomotor suppression that can be maintained for the entire stimulus duration by a 100 $\mu W/cm^2$ stimulus. Light has the ability to initiate a time-limited (30–40 min) interval of locomotor suppression (initiation effect) that can be extended by additional light (maintenance effect). Temporal integration resembling that seen in phase shifting responses to light does not exist for either phase shift or locomotor suppression responses to flashes, or for locomotor suppression responses to light pulses. We present an alternative interpretation of data thought to demonstrate temporal integration in the regulation of phase shift responses to light pulses.

Keywords

Sleep; circadian; masking; photosomnolence; locomotion; light

INTRODUCTION

One characteristic demonstrated for light-induced phase shifts is a response magnitude that varies, in sigmoidal fashion, according to the irradiance of the photic stimulus (Dkhissi-Benyahya et al., 2000; Muscat and Morin, 2005; Nelson and Takahashi, 1991). This is also true for negative masking, typically measured in nocturnal rodents as a drop in wheelrunning during exposure to light (Mrosovsky et al., 1999; Mrosovsky et al., 2000; Redlin and Mrosovsky, 1999b). For phase shifting, a similar sigmoid curve describes the

Address Correspondence to: Dr. Lawrence P. Morin, Department of Psychiatry, Stony Brook University Medical Center, Stony Brook, NY 11794-8101, phone 631-444-1613, fax 631-444-7534, lawrence.morin@stonybrook.edu.

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relationship between the duration of light exposure and shift magnitude (Mrosovsky et al., 2000). Taken together, the irradiance and duration response curves have been thought to demonstrate reciprocity with respect to the photic control of phase shift magnitude (Mrosovsky et al., 2000). Rodents appear to integrate the photic energy received over fairly long time intervals such that a long, dim stimulus and a brief, bright stimulus are expected to elicit equivalent phase shifts as long as the energies of the two stimuli are equal. This phenomenon, known as temporal integration, has been considered to be a special attribute of the circadian visual system (Nelson and Takahashi, 1991).

An effect of stimulus duration on masking has never been reported. This absence likely relates to the existence of conventional wisdom that considers negative masking to be an acute suppression of locomotion to the presence of light (Aschoff, 1981; Mrosovsky, 1999; Redlin, 2001). In fact, locomotor suppression can be initiated by millisecond ("flash") stimuli or much longer "pulse" stimuli (Morin and Studholme, 2009; van den Pol et al., 1998; Vidal and Morin, 2007). The resultant time-limited (30–40 min) locomotor suppression lasts many minutes longer than the actual stimulus and illustrates an "initiating effect" of light. The flash studies also suggest that temporal integration may not always occur in the pathway controlling either light-induced phase shifts or locomotor suppression.

The present studies were conducted to determine whether reciprocity between stimulus duration and irradiance exists for light pulse-induced locomotor suppression, just as it does to phase shifting. A second test of whether light-induced locomotor suppression obeys the principle of temporal integration was conducted with mice exposed to millisecond light stimuli. A third test was conducted to determine the relationship between irradiance and locomotor suppression that occurs during many hours of light exposure. The studies also enabled an evaluation of whether light has two distinguishable effects on locomotor suppression, one which initiates an interval of suppression and a second "maintenance effect" through which light prolongs an ongoing interval of locomotor suppression.

METHOD

Adult male C57BL J6 mice (Jackson Laboratory, Bar Harbor, ME) were housed individually in 45 L × 20 W × 20 H cm clear polycarbonate cages under a 12 hr light/12 hr dark photoperiod. Each cage contained a 16.5 cm diameter stainless steel running wheel, food in a wire cage lid hopper and a water supply. Each wheel revolution closed as microswitch, with closure detected by computer. Switch closures were recorded in 1 min bins using WinCollectRT software (written by Glenn Hudson, Electronics Shop, Stony Brook University). The same software package provided data reduction capabilities, including export of running records in raster format for figure construction or associated numerical data to spreadsheet compatible files for further analysis.

All stimuli were white light with unknown spectra. "Flash" stimuli were generated by a DynaLite Flash Head (model 2040) mounted in an animal colony room and directed at the center of animal cage rack, approximately 2 m across the room. The Dynalite Flash Head was powered by a DynaLite M1000er power supply (DynaLite, Union, NJ), the same combination employed previously (van den Pol et al., 1998; Vidal and Morin, 2007). The duration of each flash was 2 msec, as indicated by the manufacturer's specifications. Unless otherwise stated, the irradiance of each flash was approximately 3.6 J/m^2 and the animals were exposed directly to the flashes from the flash head without intervening filters, in accordance with previous procedures. When variation in flash irradiance was necessary, neutral density filters (ND; Filmtools, Burbank, CA) and a leaf diaphragm were placed in the light path. Irradiance levels for all experiments were verified with a Gigahertz-Optik P-9710 photometer (Newburyport, MA) designed to measure millisecond light stimuli over

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the spectral range of 400 to 800 nm. Light "pulses" consisted of 3 to 3000 sec presentations of a 100 watt incandescent reflector bulb (GE type 6E) with maximum irradiance of about 40 μW/cm^2 in each cage.

For each study, individual cages were placed on a rack consisting of 5 shelves, with 5 cages/shelf except for the bottom which held 4 cages. The cage rack was against a wall of an animal housing room with the light source used as the masking stimulus facing the center of the rack from the opposite side of the room. Light levels, measured immediately in front of each cage with the photodetector facing the source, did not vary by more than 2% of the value measured in front of the cage most directly in line with the light source.

Timing and number of light stimuli were computer-controlled using custom software. In addition, no attempt was made to insure that each animal was exposed to the photic stimuli in an identical manner. Thus, if an animal faced away from the light source or had its eyes closed, the actual stimulus reaching the retina would differ from the measured value and contribute to the experimental variability. In a typical experiment, the flash-control computer was set to initiate flashes at ZT13 on the current or next day. A pin photodiode detected actual occurrence of a flash or other light stimulus and, when activated, sent a signal to the data collection computer.

Subsequent to presentation of a light stimulus, the day's record of 1440 data points was exported to a spreadsheet where the data vector was reduced to include the 30 min interval before ZT13 plus the subsequent 2 hr. To be included in any analysis involving wheelrunning, an animal's data on the day of the test was required to meet three criteria: (1) during the 30 min prior to light stimulation (baseline), there could be no more than 10 zero counts (each minute with zero revolutions = 1 zero count); (2) during the 5 min prior to stimulation, there could be no more than 3 min with zero counts; and (3) the last 2 min prior to stimulation could not both have zero counts.

Experiment 1

Initial groups of mice were exposed to all combinations of the following stimuli: 3 sec, 30 sec, 300 sec or 3000 sec pulse durations; 0.01, 0.1, 1.0, 10 or 100 μW/cm^2 irradiances. Immediately after completing these tests, additional groups were exposed to all combinations of the following stimuli: 180, 300, 600 and 1200 sec light pulses with irradiances of 1.0, 10 and 100 μW/cm^2 (the results from the first tests showed that lesser irradiances produced no change in behavior). The 300 sec stimulus was included as a control and because results of the two tests with 300 sec stimuli did not differ, the data sets were combined. The corresponding stimulus energies and group sizes are shown in Table 1. Quantification of masking was achieved using the "zero count" method. This consists of counting, for each test animal that met the above criteria, the number of minutes during which the wheel revolutions were equal to zero. Zero counts were obtained across a 70 min interval beginning with the onset of the light stimulus. This interval encompassed the duration of the longest stimulus (3000 sec = 50 min) with an added 20 min to accommodate gradual, post-stimulus recovery to a baseline running level. Zero counts were also analyzed across a 40 min interval beginning when the stimulus was turned off, thus excluding the zero counts that occur during light exposure. In addition, for each animal meeting the acceptability criteria, each 5 min interval, the median wheel revolutions per minute were obtained, beginning at time zero. These values were converted to a percentage of the median revolutions per minute of the 30 min baseline and used to plot the patterns of light-induced locomotor suppression.

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Experiment 2

An energy response study was conducted using a series of ten 2 msec light flashes distributed equally across 5 min. Energy provided by each flash series was varied using a combination of leaf diaphragm and neutral density (ND) theatrical lighting gel filters (Filmtools, Burbank, CA). The Gigahertz-Optik P-9710 photometer requires flashes to be measured in Joules (J). Light energy of one J/m^2 equals the energy from a light with an irradiance of one Watt/m^2 and applied for 1 sec. The energy values were about 0.0025, 0.025, 0.25, 1.25, 2.5, 25.0 and 110.0 J/m^2 (N=32/test).

An additional group of mice (N=32) was tested along with those contributing to the energy-response study (Part A). This group received 100 flashes rather than the usual 10. This allowed a test of whether mice can integrate photic energy delivered in the form of millisecond flashes. One comparison group (Group A) received 10 flashes/5 min delivering total stimulus energy of 0.25 J/m^2 (at or just below threshold for eliciting masking). The second comparison group (Group B) also received 10 flashes, but the total energy was 2.50 J/m^2, 10 times that received by Group A; and Group C was exposed to the same total energy (2.50 J/m^2) as Group B, but the energy was delivered via 100 flashes, rather than only 10. Thus, the total energies received by Groups B and C were equal and, if energy integration occurs in response to flash stimuli, the results of these two groups should be essentially identical.

Experiment 3

Mice were given 11 hr light exposure at different irradiances (N=11–15 per test). Beginning at ZT13, animals were exposed to 1, 2.5, 5, 10 or 100 μW/cm^2 light for 11 hr. A no-light control result (N=39) was obtained for comparison. The pattern of light-induced locomotor suppression was obtained and the extent of suppression determined across the 11 hr of added light exposure.

Statistics

In nearly all instances, the data failed tests of equal variance between groups or normality of distribution. As a result, it was impossible to perform two-way analysis of variance. The non-parametric Kruskall-Wallis one-way analysis of variance by ranks was therefore applied to test main-effect differences across test conditions (effect of irradiance; effect of duration). Dunn's test was used for post-hoc analysis. Although the statistical procedures did not permit tests of interaction effects, the stimulus duration × irradiance interaction emerged naturally from the data when the units of stimulus measurement were converted to energy units. Statistics were calculated with SigmaStat v. 3.0.1 and graph generation done with SigmaPlot v. 8.02 (both from Systat Software, San Jose, CA). All graphs illustrate group medians with error bars indicating the 25th and 75th percentiles.

RESULTS

Experiment 1

The patterns of locomotor suppression following exposure to different irradiances indicate (Fig. 1A–D) that as irradiance increases, both the magnitude and duration of locomotor suppression generally increase. Exposure of mice to the highest irradiance (100 μW/cm^2) for 300 sec elicited a rapid drop in wheel running that persisted beyond the interval of light presentation (Fig. 1A). Briefer (3 or 30 sec), high irradiance stimuli likewise suppressed locomotion, but with a smaller magnitude and much shorter duration. The 3000 sec light pulse suppressed locomotion for a prolonged interval. The same general pattern occurred in response to the lesser intensities of 10 and 1 μW/cm^2 (Fig. 1B,C). Although locomotor suppression was generally greater for 100 μW/cm^2 than for 10 μW/cm^2 stimuli, the higher

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irradiance failed to elicit a significantly greater response for any stimulus duration. Stimuli at 0.1 (Fig. 1D) or 0.01 $\mu W/cm^2$ (data not shown) neither suppressed nor increased locomotion. These data have been excluded from further presentation.

Quantitative analysis of the zero counts summed over a 70 min interval showed (Fig. 2) that the level of locomotor suppression increased significantly as irradiance increased, even within the 3 sec stimulus condition ($p<.01$). Locomotor suppression also increased with stimulus duration, even within the 1 $\mu W/cm^2$ condition ($p<.02$). Combination of duration and irradiance into a stimulus energy (J/m^2) variable permitted a single plot describing the relationship between light energy and zero counts (i.e., locomotor suppression). It shows that the locomotor suppression increases as energy exposure increases (Fig. 3). A standard curve-fitting procedure (Nelson and Takahashi, 1991) produced a smooth curve linear over about 2 orders of magnitude.

The fitted curve (Fig. 3) obscures an important characteristic of the data. In reality, the manner of locomotor suppression by the 1200 and 3000 sec stimuli was qualitatively different from manner of responses to briefer stimuli. In response to stimuli which were 30–600 sec, suppression of locomotor activity persisted for a prolonged interval after stimulus termination (Fig. 4). Post-stimulus persistence of locomotor suppression was significantly increased by higher irradiances for each duration tested, but the increase was not linearly related to duration. Instead, the post-stimulus persistence significantly declined after the longest stimuli. The locomotor suppression response to the 30 sec, 100 $\mu W/cm^2$ stimulus was about 55 times longer than the stimulus itself.

Experiment 2

The energy response test to 10 light flashes yielded a significant effect of light energy on locomotor suppression ($p<.001$). The median zero minutes in response to 0.0025, 0.025, 0.25, 1.25, 2.50, 25.0 or 110 J/m^2 were 10.0, 11.0, 13.0, 26.0, 27.5, 36.0 and 45.0 per group, respectively. The two lowest energies did not elicit responses different from no light controls (median=8.0) or from the effects of the third lowest stimulus energy. The 1.25 J/m^2 stimulus elicited a response that differed from that caused by the highest energy and the latter group did not differ from the groups receiving the two next highest energies. The four strongest energies elicited responses that differed from each of the three lowest.

The test of temporal integration showed that locomotor zero minutes varied significantly according to treatment ($p<.001$). The 10 flash/2.50 J/m^2 stimulus series greatly increased locomotor suppression compared to the 10-fold less energetic 10 flash/0.25 J/m^2 stimulus (Fig. 5). More importantly, a 2.50 J/m^2 stimulus presented as 100 flashes yielded locomotor suppression that was significantly less than that induced by the 10 flash/2.50 J/m^2 stimulus series ($p<.05$).

Experiment 3

Exposure to light for 11 hr elicited locomotor suppression that varied in magnitude with stimulus irradiance (Fig. 6). Analysis of the zero count data revealed a significant treatment effect ($p<.001$). Control mice in the dark spent 57% of their minutes without generating one or more wheel revolutions. As a result, the zero count measure was less sensitive than total wheel revolutions during the 11 hr interval. Wheel revolutions by groups exposed to >2.5 $\mu W/cm^2$ during the 11 hr light were significantly reduced compared to controls (Fig. 6C).

DISCUSSION

The magnitude of wheel running suppression is related to irradiance during a standard light pulse (Mrosovsky et al., 1999; Redlin and Mrosovsky, 1999b). The present studies confirm

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and extend that result using a variety of stimulus durations. The results of stimulus duration tests support the view that light has two effects on locomotor suppression. The first is an initiating action that leads to rapid locomotor suppression which persists for a 30–40 min interval in the absence of additional light (Morin and Studholme, 2009; Vidal and Morin, 2007). The second is a maintenance action that prolongs already induced locomotor suppression (present data; Morin and Studholme, 2009). The results also indicate an absence of temporal integration for light-induced locomotor suppression.

Locomotor suppression, photosomnolence and masking

The term, "negative masking," has been frequently used in reference to the suppressing effect of light on nocturnal wheelrunning in mice and hamsters (Mrosovsky, 1999; Mrosovsky et al., 1999; Redlin, 2001; Redlin and Mrosovsky, 1999b). In the current presentation, the decrease in the amount and pattern of nocturnal wheelrunning was employed as an index of how light alters function of the non-image forming visual system. The extent of light-induced locomotor suppression is presumed to be a index correlated with the function of an underlying mechanism causal of locomotor suppression, but not pointing directly at it. In the case of light-induced locomotor suppression as assessed in the present studies, the decline in activity (wheelrunning or open field) is likely secondary to the induction of quiescence and sleep, together referred to as "photosomnolence" (Morin and Studholme, 2009).

Light initiation of locomotor suppression

The typical test of light's ability to suppress locomotion has utilized 1 hr stimuli (e.g., (Mrosovsky et al., 1999; Redlin and Mrosovsky, 1999b)). Results of such studies have fostered the idea that locomotor suppression is contingent upon the continued presence of light (Mrosovsky, 1999; Redlin, 2001). It is now apparent, however, that a few millisecond light flashes are sufficient to elicit locomotor suppression which persists for a fairly long interval (30–40 min) (Morin and Studholme, 2009; Vidal and Morin, 2007). Such results are consistent with those reported here and emphasize that light has an acute ability to *initiate* an interval of locomotor suppression. Additionally, the results obtained with flashes also demonstrate that the sequence of events initiated by light persist to completion without the necessary presence of additional light. An equivalent interval of locomotor suppression also occurs in response to bright light pulses that are 30–600 sec duration (present data). Thus, light initiates a time-limited series of events associated with a state change from highly active to quiescent to sleeping, with sleep beginning about 10 min after light onset and lasting about 25 min (Morin and Studholme, 2009).

Light maintenance of locomotor suppression

Light can also *maintain* an already ongoing interval of locomotor suppression. As previously demonstrated, an interval of locomotor suppression induced by a 5 min pulse can be extended (i.e., the existing state of locomotor suppression is maintained) by a single flash administered 25 min later (Morin and Studholme, 2009). In contrast, locomotor suppression cannot be initiated by a single flash (or even 5 flashes, under some circumstances) (Morin and Studholme, 2009). The ability of a single flash to maintain, but not initiate, an interval of locomotor suppression distinguishes between the two effects of light. Moreover, extension of the locomotor suppression is directly related to the number of flashes received, whereas duration of locomotor suppression induced by flashes appears to be an all-or-none response to flash number.

The present observation that 30–600 sec pulse stimuli yield equivalent length intervals of locomotor suppression contrasts with the ability of much longer light stimuli to greatly lengthen the duration of locomotor suppression. It is possible that the longer light

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presentations repeatedly elicit the same photosomnolence sequence as elicited by millisecond flashes thereby maintaining locomotor suppression. For a long-lasting light stimulus, this would mean that with each additional instant of the stimulus, the photosomnolence interval would be re-initiated. If this is the case, then the last 30 sec (from Fig. 4) of a long stimulus would be expected to yield the same duration of locomotor suppression as occurs following a single 30 sec pulse stimulus. Instead, the results show that locomotion suppression is prolonged for less time after long stimuli (e.g., 3000 sec) than after shorter stimuli. It is more likely that the maintenance effect is contingent upon the actual presence of light. The extent to which the animals are actually asleep during prolonged light exposure is not known.

Temporal integration and light-induced locomotor suppression

The essential idea behind temporal integration is that a bright light of short duration can induce a behavioral change equal to that induced by a dim light of long duration, as long as the two stimuli have equal total energy (Nelson and Takahashi, 1991). The circadian visual system is presumed to estimate and respond to the stimulus energy. The present investigations applied two methods to determine whether the system regulating light-induced locomotor suppression demonstrates temporal integration.

The first approach evaluated the effects of stimulus irradiance and duration and found suppression to increase with irradiance in a manner consistent with the existing literature for both locomotor suppression and phase shifting (Mrosovsky et al., 1999; Nelson and Takahashi, 1991; Redlin and Mrosovsky, 1999b). Unlike the effect of irradiance, the stimulus duration effect on locomotor suppression is much less clear. This results, in part, because light both initiates and maintains the ongoing suppression. As a consequence of the initiating action, pulse stimuli ranging from 30 to 600 sec induce essentially the same duration response, most of which occurs in the absence of light (Fig. 4) (Morin and Studholme, 2009). Most importantly, irradiance and duration do not interact to yield longer intervals of locomotor suppression. This is especially evident in Fig. 2 in which there are no significant within group increases in locomotor suppression as the stimulus irradiance increases from 10 to 100 $\mu W/cm^2$. With the 1200 and the 3000 sec stimuli, the effect of duration is largely limited to the interval during which light is actually present and post-stimulus persistence of locomotor suppression is less.

When the stimulus irradiance and duration variables are merged into an energy index (J/m^2), a plot of the data (Fig. 3) is approximately sigmoidal and comparable to previous presentations (Nelson and Takahashi, 1991). However, it is not clearly sigmoidal and, if the data from Experiment 3 were added (11 hr of almost complete locomotor suppression), the energy response curve would not show saturation and lose all semblance of being sigmoidal. Rather, for each minute of additional light exposure, animals would show one minute of locomotor suppression regardless of the accumulated energy exposure. Thus, the conclusion must be that temporal integration does not exist in the photic input pathway mediating locomotor suppression because of the absence of reciprocity between stimulus irradiance and duration.

The second experimental approach to temporal integration employed 2 msec light flashes. In this test, two flash series having equal energy but different numbers of flashes failed to demonstrate equal levels of locomotor suppression. Again, the results are consistent with the view (Morin and Studholme, 2009) that mice do not employ normal temporal integration when determining the extent of locomotor suppression in response to a series of flashes and with the more general view that temporal integration is not involved in light-induced locomotor suppression.

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Circadian rhythm phase and locomotor suppression are similarly regulated by light

Phase shift responses to light energy, either expressed as an estimate of photon number or an energy measure, are thought to show temporal integration (Dkhissi-Benyahya et al., 2000; Muscat and Morin, 2005; Nelson and Takahashi, 1991). Both phase shift and locomotor suppression responses are mediated by a similar photic input pathway involving classical and non-image forming photoreception (by ipRGCs bearing melanopsin photopigment) (Altimus et al., 2009; Hattar et al., 2003; Mrosovsky et al., 2001; Mrosovsky and Hattar, 2003; Thompson et al., 2008). Each response remains relatively intact after elimination of either classical or ganglion cell photoreception. Simultaneous elimination of both classical and non-image forming photoreception or physical destruction of the ipRGCs eliminates both phase shifting and light-induced locomotor suppression (Hattar et al., 2003). The current interpretation is that rod photoreception passes photic input to ipRGCs which serve as the conduits for transmittal of rod and ipRGC input to the brain (Altimus et al., 2009; Gõz et al., 2008; Guler et al., 2008; Hatori et al., 2008).

Photic input entering the SCN controls circadian rhythm phase (Johnson et al., 1988). However, the site at which photic information acts to suppress locomotion or induce photosomnolence (if a mechanistic distinction exists between the two behavioral changes) is not certain. One investigation shows that SCN lesions abolish light-induced locomotor suppression (Li et al., 2005), while another suggests that such suppression continues (Redlin and Mrosovsky, 1999a). Absence of intact retinal projections to the SCN eliminates both entrainment and any indication that photoperiod modulates locomotion (Johnson et al., 1988). Likewise, when circadian rhythmicity is restored in arrhythmic animals after an SCN transplant, there is no indication of photoperiod-induced locomotor modulation (Lehman et al., 1987). Thus, both light-induced phase shifting and locomotor suppression appear to be mediated by the same pathway to the SCN, although the possibility that locomotor suppression is mediated by retinohypothalamic input to areas caudal to the SCN cannot be completely excluded at this time.

As indicated above, the present data do not provide support for the view that temporal integration is an operational principle regulating light-induced locomotor suppression. Rather, the results are consistent with previous data showing that neither phase shifts nor locomotor suppression systematically increase with the number of flashes (Vidal and Morin, 2007). The data from Expt. 2, which indicate a failure of equally energetic stimuli to elicit equal responses, are also very similar to those obtained in a previous study in which the measure was phase shift magnitude (Vidal and Morin, 2007). Thus, with one exception, all tests of temporal integration yield similar results for both phase shifting and locomotor suppression. The sole exception is the purported effect of stimulus duration on phase shift magnitude (Nelson and Takahashi, 1991). This exception bears further scrutiny because the published phase shift data indicate that the circadian system is relatively less sensitive to long (3600 sec) stimuli than it is to 300 sec stimuli (Nelson and Takahashi, 1991), an observation consistent with the present data showing that locomotor suppression responses to long, but not the short, stimuli involve both initiating and maintenance effects of light.

A false assumption may underlie phase shift tests of temporal integration

If the photic input pathways for phase shifting and locomotor suppression are the same; if the effects of stimulus irradiance on the two responses are similar; and if the effects of light flashes on the two responses are the same (with temporal integration absent), then why is temporal integration applicable to light pulse effects on phase shifts, but not to light-induced locomotor suppression?

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A parsimonious answer is that temporal integration does not exist for either behavioral effect of light. This explanation can be true if the major assumption underlying tests of temporal integration can be rejected. The critical assumption is that the light stimulus is uniformly effective across its entire duration such that every instant of light exposure has an effect equivalent to that of every other instant, the sum of these exposures being the integrated stimulus. For locomotor suppression, light effects are not uniform. as indicated by the distinction between its initiation and maintenance actions.

The fact that certain series of millisecond flashes, but not others, elicit full size phase shifts in both hamsters and mice, indicates a strong likelihood that the assumption of functional stimulus uniformity is incorrect. This conclusion, in conjunction with absence of reciprocity between the number of flashes and energy of those flashes, has two major implications. The first is that *both* locomotor suppression and phase shifting may be responses to a light-initiated event sequence that runs to completion without the need for further light. The second implication is that additional light would be expected to augment locomotor suppression because of its maintenance action, but this *cannot* occur for phase shifts because they are constrained by the very nature of the circadian clock mechanism. The phase response curve, derived from operation of that mechanism, describes severe limits on shift direction and magnitude (Daan and Pittendrigh, 1976). These limits appear to occur downstream of the point at which the photic input path controlling phase shifts diverges from that controlling locomotor suppression. Exactly how and where this divergence occurs remains to be determined.

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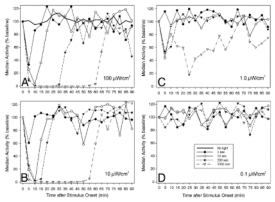

Figure 1.

Running patterns of mice following light exposure for 3, 30, 300 or 3000 sec at (A) 100 μW/cm^2; (B) 10 μW/cm^2; (C) 1 μW/cm^2; and (D) 0.1 μW/cm^2. Plot (A) also includes results for an no-light control condition (solid line without symbol; N=24). The data obtained with 0.01 μW/cm^2 light are not shown, but were similar to those exposed to 0.1 μW/cm^2. The median wheel revolutions during each 5 min interval is shown as a percentage of baseline wheel revolutions. Time 0 = onset of the light stimulus. The legend in (D) applies to panels (A–D).

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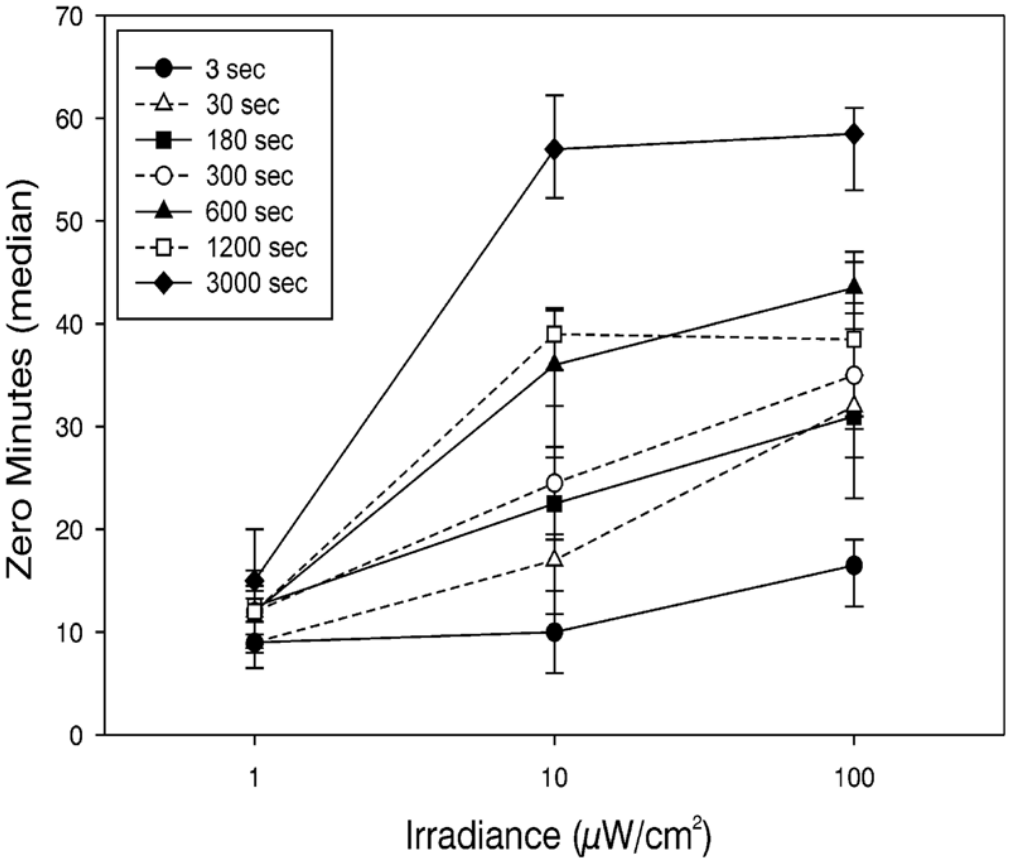

Figure 2.

Locomotor suppression, calculated as zero minutes over a 70 min interval beginning with stimulus onset varies with both irradiance and duration. Results for all combinations of stimulus irradiance and duration are shown except those involving irradiances of 0.1 or 0.01 $\mu W/cm^2$ which failed to induce locomotor suppression regardless of duration. Numbers indicate stimulus durations in seconds.

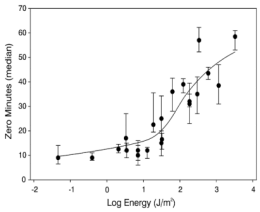

Figure 3.
Zero minutes from Fig. 2 plotted relative to stimulus energy (J/m^2). The solid line represents the best fit described by a 4 parameter logistic equation based on the Marquardt-Levenberg algorithm (SigmaPlot v. 9.02).

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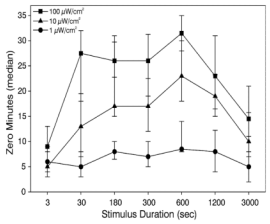

Figure 4.

Effect of stimulus duration on locomotor suppression as measured by the zero counts during a 40 min interval beginning at the termination of the light stimulus. The figure shows that post-stimulus locomotor suppression is greater with higher irradiances, but long stimuli tend to induce less post-stimulus suppression than shorter stimuli.

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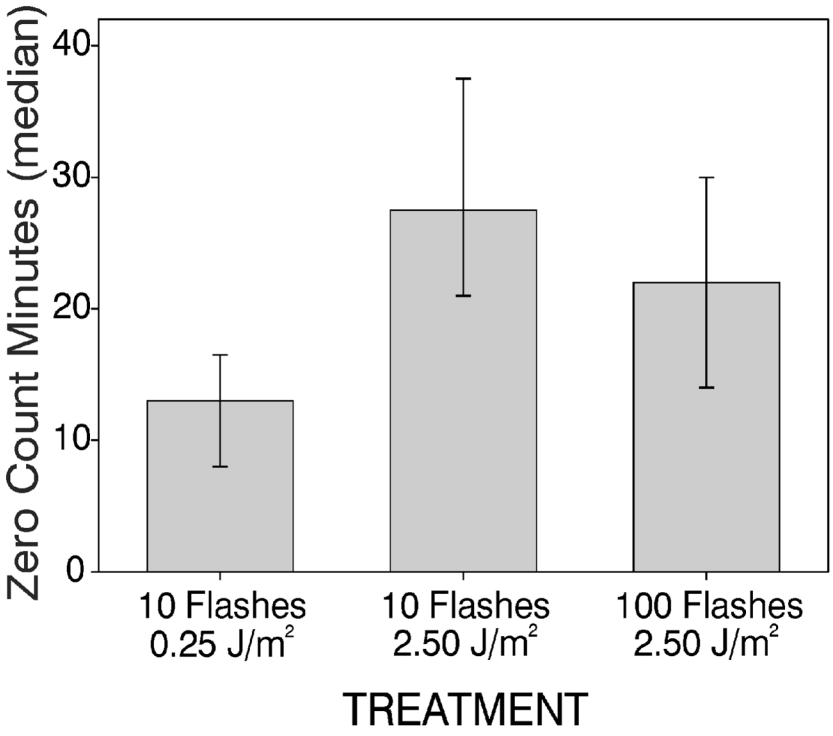

Figure 5.
Locomotor suppression in response to 100 flashes is not the same as the response to 10 flashes despite the two flash series having equal energy indicating that normal photon integration is absent. Treatment labels indicate the number of flashes and total energy received. Each group differs significantly from the other groups.

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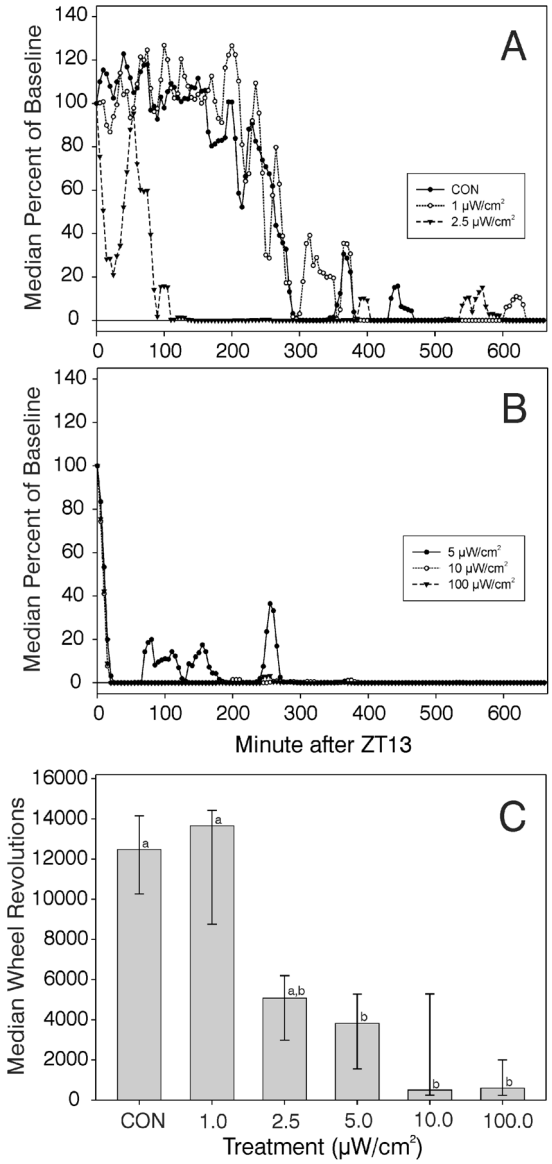

Figure 6.
Locomotor activity remains suppressed in irradiance-dependent fashion by light administered across the night. (A,B) Patterns of locomotor activity in response to difference irradiances which lasted 11 hr beginning at ZT13. (C) Wheel revolutions in response to the various irradiances. Groups bearing common letter identifiers do not differ.

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Table 1

Energy (J/m^2) delivered by each stimulus in Experiment 1

Duration (sec)	**Irradiance[a] (N/group)**				
Test Series 1	**0.01**	**0.1**	**1**	**10**	**100**
3	0.001 (32)	0.005 (25)	0.046 (17)	0.312 (30)	3.902 (20)
30	0.010 (28)	0.044 (33)	0.395 (14)	3.323 (25)	32.187 (28)
300	0.088 (21)	0.392 (19)	3.506 (31)	31.294 (30)	296.33 (33)
3000	0.953 (29)	3.132 (33)	31.172 (37)	335.13 (27)	3258.9 (26)
Test Series 2					
180	--	--	2.079 (16)	18.812 (16)	183.78 (13)
300	--	--	3.341 (15)	31.301 (23)	305.41 (12)
600	--	--	7.095 (14)	62.341 (16)	599.58 (14)
1200	--	--	12.794 (17)	124.08 (17)	1158.00 (18)

[a] (μW/cm^2)