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DRUGS THAT PREVENT MOUSE SLEEP ALSO BLOCK LIGHT-INDUCED LOCOMOTOR SUPPRESSION, CIRCADIAN RHYTHM PHASE SHIFTS AND THE DROP IN CORE TEMPERATURE

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Abstract—Exposure of mice to a brief light stimulus during their nocturnal active phase induces several simultaneous behavioral or physiological responses, including circadian rhythm phase shifts, a drop in core body temperature (Tc), suppression of locomotor activity and sleep. Each response is triggered by light, endures for a relatively fixed interval and does not require additional light for expression. The present studies address the ability of the psychostimulant drugs, methamphetamine (MA), modafinil (MOD) or caffeine (CAF), to modify the light-induced responses. Drug or vehicle (VEH) was injected at CT11 into constant dark-housed mice then exposed to 5-min 100 μW/cm^2 light or no light at CT13. Controls (VEH/Light) showed approximately 60-min phase delays. In contrast, response was substantially attenuated by each drug (only 12–15 min delays). Under a 12-h light:12-h dark (LD12:12) photoperiod, VEH/light-treated mice experienced a Tc drop of about 1.3 °C coincident with locomotor suppression and both effects were abolished by drug pre-treatment. Each drug elevated activity during the post-injection interval, but there was also evidence for CAF-induced hypoactivity in the dark prior to the photic test stimulus. CAF acutely elevated Tc; MA acutely lowered it, but both drugs reduced Tc during the early dark (ZT12.5–ZT13). The ability of the psychostimulant drugs to block the several effects of light exposure is not the result of drug-induced hyperactivity. The results raise questions concerning the manner in which drugs, activity, sleep and Tc influence behavioral and physiological responses to light.

Key words: arousal, suprachiasmatic, photosomnolence, thermoregulation, circadian, masking.

INTRODUCTION

The light-type phase response curve describes the relationship between an organism's rhythm phase shift magnitude and the circadian time at which the stimulus occurred (Daan and Pittendrigh, 1976). In general, light exposure early in the subjective night elicits phase delays and phase advances occur if exposure occurs later. Light at night coincidentally suppresses locomotion in nocturnal rodents, a phenomenon labeled 'negative masking' (Mrosovsky, 1999). Such light-induced locomotor suppression occurs rapidly and is a prelude to another consequence of light exposure, namely, photosomnolence or light-induced sleep (Morin and Studholme, 2009; Morin et al., 2010).

Several studies suggest that reduced or absent locomotor activity may be necessary for normal, light-induced phase shifts (Ralph and Mrosovsky, 1992; Mistlberger and Antle, 1998; Mistlberger and Holmes, 1999; Edelstein et al., 2003). In addition to its locomotion suppression effects, light also induces a large decline in core body temperature (Tc; Studholme et al., 2013). Tc is normally lowest during the daylight hours when most nocturnal species sleep and, regardless of time of day, is lower during sleep than during wake (Obal et al., 1985).

Methamphetamine (MA), modafinil (MOD) and caffeine (CAF) are well known as sleep-prevention pharmaceuticals (see (Edgar and Seidel, 1997) for a discussion). MA maintains wakefulness and induces hyperactivity (Edgar and Seidel, 1997). MOD is used clinically to maintain wakefulness, in part, because it induces less activity than MA (Edgar and Seidel, 1997; Okuro et al., 2010). CAF has more limited effects, but modestly increases locomotor activity (Okuro et al., 2010). In addition, certain doses of each drug are reported to increase Tc (Edgar and Seidel, 1997; Okuro et al., 2010; Phelps et al., 2010).

MA treatment has also been shown to block both light-induced circadian rhythm phase shifts and FOS protein in suprachiasmatic nucleus (SCN) neurons (Moriya et al., 1996; Ono et al., 1996). Similar effects were not found after MOD treatment (Webb et al., 2006). The effect of CAF on either of these responses has not been reported, although the drug attenuates phase shifts induced by wheel running (Antle et al., 2001). Further, the adenosine R1 receptor, a native target for CAF, has been implicated as a modulator of light-induced rhythm shifts, FOS in the SCN, as well as SCN field potential

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Abbreviations: ANOVA, analysis of variance; CAF, caffeine; DA, dopamine; DD, constant dark; DMSO, dimethyl sulfoxide; DSI, Data Sciences International; IP, intraperitoneal; LD12:12, 12-h light:12-h dark; LED, light emitting diodes; MA, methamphetamine; MOD, modafinil; SCN, suprachiasmatic nucleus; SNK, Student–Newman–Keuls; Tc, core body temperature; VEH, vehicle.

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amplitude following optic nerve stimulation (Watanabe et al., 1996; Elliott et al., 2001; Sigworth and Rea, 2003). CAF may also induce phase delays by direct effect on the SCN (Ding et al., 1998).

We have suggested elsewhere that light-induced circadian rhythm phase shifts, locomotor suppression/photosomnolence and reduction of body temperature may be controlled via a common retinal input pathway (Morin, 2013b; Studholme et al., 2013). The present studies were designed to determine whether the three psychostimulant drugs are able to prevent these responses with the expectation that if a drug blocked one of the responses to light, it would block them all. Moreover, because of their similar sleep-prevention attributes, the three drugs were predicted to have similar effects on the light-induced responses despite their differing modes of action and differing effects on general locomotion.

EXPERIMENTAL PROCEDURES

Animals and housing conditions

Male C57BLJ/6 mice (Jackson Laboratory, Bar Harbor, ME, USA) were initially housed individually in polycarbonate test cages (45 L × 20 W × 20 H cm). During Experiments 1–3, each cage contained a 16.5-cm diameter stainless steel running wheel and was semi-isolated, along with 4–5 other cages, in an enclosed, light-tight shelf on a rack consisting of five such shelves. During Experiments 4–6, the test cages were on stainless steel shelving in a standard animal housing room. Mice were maintained under a 12-h light:12-h dark (LD12:12) photoperiod (light on at 2 AM clock time), except as otherwise described. Ambient temperature was 21 ± 1 °C; food and water were available *ad libitum*.

Lighting of each shelf in the semi-isolation chamber consisted of a linear array of 48 broad spectrum white light light emitting diodes (LEDs) (LBFA-CW12, Superbrightleds.com) arranged approximately 10 cm from the wheel end of the cage. Irradiance within each chamber was controlled by an eight bit D–A voltage controller, an LED dimmer (OSRAM OT DIM, Osram Sylvania, Danvers, MA, USA) and custom software (LightControl written by Glenn Hudson, Stony Brook University) that allowed timing of the LED light with one second accuracy. In Experiments 1–3, the LED light turned on (irradiance = 100 μW/cm^2) at the pre-programmed time and remained on for 5 min. In Experiments 4–6, a 5-min, 100 μW/cm^2 light pulse was also used, but was delivered from a 150-W incandescent light source (#120-P38BI-1, Cheaplights.com).

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Stony Brook University (NY) and conducted in accordance with the United States National Institutes of Health Guidelines regarding the care and use of animals for experimental procedures.

Data recording and analysis

Each revolution of a running wheel closed a microswitch. Such closures were recorded as revolutions per minute by a data acquisition system (WinCollectRT software written by Glenn Hudson, Stony Brook University). The software also created the raster format representation of the daily locomotor activity of each animal and exported the data to a spreadsheet for further analysis. Independent groups of mice were tested in each experiment.

Experiments 1–3. Mice stably entrained to LD12:12 were exposed to constant dark (DD). After 5 days in DD, a line was eye-fitted through the daily wheel-running onsets in DD and extrapolated to day 6. The light pulse was delivered to each shelf at the clock time that would effectively stimulate the largest number of mice on that shelf. Effectively stimulated mice were those that received light exposure during the interval of CT13–CT14 and only these were used in the subsequent analysis. An eye-fitted line through five daily, post-stimulus activity onsets was extrapolated back to day 6 and the phase shift (time difference between the extrapolated pre- and post-test activity onsets) was measured on that day with a digital caliper.

For each of these experiments, a modification of a previous procedure (Morin and Studholme, 2009), provided a ''wheel-running suppression index.'' This measure was calculated as the percentage of minutes in a 60-min interval after stimulus onset in which the animal registered zero wheel revolutions.

Experiments 4–6. Mice were housed under LD12:12, then deeply anesthetized with a mixture of ketamine (100 mg/kg, Butler Supply, Dublin, OH, USA) and xylazine (10 mg/kg, Lloyd Laboratories, Shenandoah, IA, USA) and intra-abdominally implanted with a telemetric transmitter (PhysioTel model TA-11-F10; Data Sciences International (DSI), St. Paul, MN, USA) that simultaneously obtained temperature and activity data. ART v4.3 (DSI) software was used for data collection. Animals were allowed to recover from surgery for at least 8 days prior to participation in an experiment.

Analysis and statistics

Activity indices obtained from the DSI transmitters and Tc data were graphed for each animal using SigmaPlot 11.0 (Systat Software, San Jose, CA, USA). On the average, 2 units of DSI activity correspond to approximately 1-cm distance moved (Studholme et al., 2013).

Analysis of the effect of light on Tc was accomplished by evaluating change in Tc calculated by subtracting the lowest Tc measured during a 20-min interval beginning at the time of light stimulus onset from the average Tc during the 20 min prior to stimulus onset time. Light-induced change in activity level was obtained by subtracting each animal's post-stimulus activity level from its average activity level during the 20 min prior to stimulus onset. Because there were large minute-to-minute fluctuations in the activity levels, the actual post-stimulus activity index subtracted was the level represented by the first quartile (25th percentile) of all activity across the 20-min test interval after stimulus onset.