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BRIGHT ARTIFICIAL LIGHT SUBSENSITIZES A CENTRAL MUSCARINIC MECHANISM

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Summary

Supersensitivity of a muscarinic mechanism is implicated in the pathophysiology of depression. Bright artificial light is efficacious in the treatment of Seasonal Affective Disorder (SAD). We studied the effect of constant bright light (11,500 lux) on the sensitivity of adult, male rats to oxotremorine, 1.5 mg/kg ip, using a repeated measures design. Oxotremorine challenges were proceeded by the injection of methylscopolamine, 1 mg/kg ip, by 30 minutes. Temperature was telemetrically measured every 10 minutes for 120 minutes starting 10 minutes after the injection of oxotremorine. Prior to and after 7 continuous days of exposure to bright light, the sample exhibited a hypothermic response of 2.50 $\pm$ 0.48°C (mean $\pm$ SEM) and 0.29 $\pm$ 0.31°C (mean $\pm$ SEM), respectively ($p < 0.0014$). All 7 animals exhibited blunting to the thermic response to oxotremorine. Bright light also blocked the capacity of amitriptyline to supersensitize a central muscarinic mechanism. Exposure to light at an intensity of 300 lux for 7 days had no effect on the thermic response to oxotremorine. These data are consistent with the hypotheses that the biology of depression involves supersensitivity of central muscarinic mechanisms and that the effects of bright artificial light are not the consequence of shifting circadian rhythms.

Seasonal Affective Disorder (SAD) is a syndrome characterized by recurrent depressions which occur annually (1). This syndrome responds to daily treatment with 2-6 hours of bright artificial light (1-5). A mechanism accounting for the efficacy of this treatment has not been identified. Certain forms of affective disorders may involve state independent supersensitivity of central cholinergic systems (6). Data supporting a cholinergic hypothesis of depression were recently summarized (7-8). This article presents data indicating that bright artificial light blunts the hypothermic response to oxotremorine and prevents the supersensitization of muscarinic mechanisms produced by a muscarinic receptor antagonist (9).

Materials and Methods

The dependent variable in the experiments reported here was change in core temperature in response to a muscarinic agonist, oxotremorine. Core

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temperature was measured using a telemetric thermosensor, the Model VM Mini-Mitter (Mini-Mitter Corp., Sun River, OR). These devices emit radio waves at a frequency which is detectable using an AM receiver. Information regarding the calibration, reliability and validity of the Mini-Mitter is available elsewhere (10).

All oxotremorine challenges started at between 0900 and 1000 on the first and eighth days of Experiment 1 and on the first, eighth and fifteenth days of Experiments 2 and 3. Challenges occurred 20 hours after the fourteenth and twenty-eighth doses of amitriptyline (AMI) in Experiments 2 and 3.

Amitriptyline (AMI) hydrochloride and oxotremorine (base) was purchased from Sigma Chemical Co. (St. Louis, MO).

Full-spectrum bright artificial light (11,500 lux) was emitted from a bank of 8 122 cm long Vitalight tubes suspended 50 cm above the animals. This light unit (Duro Test Co., Bergen, NJ, Model 5599) is used to treat patients with SAD (2). Temperature under the lights was 23°C.

Experiment 1: Mini-Mitters were implanted into the peritoneal cavities of 7 adult, male Sprague Dawley rats (mean weight $\pm$ SEM = 219.3 $\pm$ 9.2 g). Experiment 1 involved measurement of the hypothermic response to oxotremorine, 1.5 mg/kg ip, prior to exposure to bright artificial light (baseline), after one week of constant light exposure, and one week after the withdrawal of bright artifical light. During the baseline and withdrawal phases the animals were subject to the standard 12 hour light/dark cycle.

Methylscopolamine nitrate, 1 mg/kg ip, was administered 30 minutes prior to the injection of oxotremorine in order to block peripheral effects of the muscarinic agonist. Core temperature was measured 30 minutes after the injection of methylscopolamine nitrate. The dependent variable was change in core temperature relative to this point in time. Oxotremorine, 1.5 mg/kg ip, was then administered and core temperature measured every 10 minutes for 120 minutes. We previously reported that methylscopolamine nitrate, 1 mg/kg ip, does not alter core temperature 30 minutes after injection, relative to the pre-injection baseline (11).

Experiment 2: Mini-Mitters were implanted in the peritoneal cavities of 8 adult, male Sprague-Dawley rats (mean weight $\pm$ SEM = 339.5 $\pm$ 12.1 g). The hypothermic response to oxotremorine, 1 mg/kg ip, was first measured at baseline (prior to any experimental manipulation). This challenge was immediately followed by one week of treatment with AMI, 10 mg/kg ip at 0900 and 1700, under standard lighting conditions. The first week of treatment with AMI was followed by an oxotremorine challenge. At the conclusion of this challenge the animals were immediately treated with bright artificial light for 7 consecutive days. AMI, 10 mg/kg ip twice daily, was administered throughout this period. At the conclusion of this phase of light exposure, the animals were rechallenged with oxotremorine (while still under the lights). Finally, the animals were returned to standard vivarium conditions. Treatment with AMI continued. A fourth oxotremorine challenge was conducted 7 days later.

Experiment 3: Mini-Mitters were implanted into the peritoneal cavities of 10 adult, male Sprague Dawley rats (mean weight $\pm$ SEM = 223 $\pm$ 6.3). The hypothermic response to oxotremorine, 1 mg/kg ip, was measured at baseline. This was followed by one week of treatment with AMI, 10 mg/kg ip, twice

daily under standard lighting conditions. The first week of treatment with AMI was followed by an oxotremorine challenge. The animals were then treated with normal flourescent light at an intensity of 300 lux for 7 consecutive days. AMI, 10 mg/kg twice daily, was administered throughout this period. At the conclusion of this, the second week of AMI administration, the animals were rechallenged with oxotremorine while they continued to be exposed to light at an intensity of 300 lux.

The authors previously established that AMI, 10 mg/kg ip twice daily, produces supersensitivity of a central muscarinic mechanism (11). The capacity of this agent to do this displays dose-dependence (12). Thus, demonstration that bright artificial light prevents the development of supersensitivity to oxotremorine would indicate that it is a potent inhibitor of the development of supersensitivity of a central muscarinic mechanism.

Significance of change in the thermic responsiveness of each individual animal between weeks was measured using the Student's paired t-test. This was possible because the core temperature of each animal was measured 12 times after the injection of oxotremorine. Significance of the mean change in core temperature between points in time for each sample was also determined using the paired t-test. All measures of variance in the text refer to the standard error of the mean (SEM).

Results

Experiment 1: Mean core temperature was 37.5 ± 0.29°C prior to the first challenge with oxotremorine. Mean core temperature was 37.8 ± 0.30°C in the presence of bright light following one week of exposure to bright artificial light ($p > 0.15$, $t = 1.64$, $df = 6$). Table 1 summarizes the mean thermic response over the 12 time points for all 7 animals. Six (6) animals demonstrated significant blunting of the hypothermic response to oxotremorine (1.5 mg/kg ip) at $\alpha < 0.0001$ after light treatment. Further, the mean hypothermic response of the sample, -2.46 ± 0.41°C prior to light treatment and -0.36 ± 0.31°C afterwards, was significantly blunted after chronic light exposure ($p < 0.0007$, $t = 6.50$, $df = 6$). One week after the discontinuation of light treatment the mean hypothermic response of the sample increased to -3.14 ± 0.23°C ($p < 0.00009$, $t = 6.16$, $df = 6$).

TABLE I

Animal #	A Mean Hypothermic Response at Baseline	B Mean Hypothermic Response After One Week of Light Treatment	C Probability Based on Paired t-test A vs B p <
1	-1.93 ± 0.33	-0.41 ± 0.17	0.00003
2	-2.85 ± 0.44	-0.24 ± 0.17	0.00004
3	-1.55 ± 0.39	-1.11 ± 0.17	n.s.
4	-2.98 ± 0.39	-0.45 ± 0.11	0.000001
5	-4.04 ± 0.75	-1.27 ± 0.20	0.0001
6	-0.82 ± 0.34	+1.28 ± 0.20	0.000005
7	-3.07 ± 0.40	-0.33 ± 0.46	0.000004
	-2.46 ± 0.41	-0.36 ± 0.31	0.0007

The hypothermic response of individual animals ($n = 7$) to oxotremorine (1.5 mg/kg ip) at baseline (pretreatment) and after 7 days of 11,500 lux light treatment.

Experiment 2: The mean core temperature of the sample prior to the first oxotremorine challenge was 37.6 ± 0.13°C. Mean core temperature was also 36.6 ± 0.24°C in the presence of bright light following one week of phototherapy ($p = 1$, $t = 0$, $df = 7$). Table 2 summarizes the mean thermic response for each individual animal for the three phases of the experiment (baseline, treatment with AMI, and concurrent treatment with AMI and bright light).

The sample exhibited a significant hypothermic response to oxotremorine, 1.0 mg/kg ip, of -1.19 ± 0.12°C at baseline ($p < 0.00003$, $t = 9.93$, $df = 7$). Following one week of treatment with AMI, 4 animals exhibited supersensitivity to oxotremorine at $\alpha < 0.02$. Further, this treatment increased the sample's hypothermic response to -1.68 ± 0.21°C ($p < 0.04$, $t = 2.55$, $df = 7$).

All 8 animals exhibited a reduction in their hypothermic response relative to baseline, after one week of treatment with both AMI and bright light ($p = 0.0039$, sign test). Six (6) of 8 animals exhibited significant blunting of their hypothermic responses at $\alpha < 0.002$ and another animal demonstrated a trend towards significance. The difference in the mean responses to oxotremorine at baseline (-1.19 ± 0.12°C) and after concurrent treatment with AMI and light (-0.22 ± 0.25°C) was highly significant ($p < 0.005$, $t = 4.17$, $df = 7$).

TABLE II

Animal #	Mean Hypothermic Response at Baseline	Mean Hypothermic Response, 1 Week of AMI Treatment	Mean Hypothermic Response, 2 Weeks AMI + 1 Week Light	$p <$ Baseline vs 1 Week AMI	$p <$ Baseline vs 1 Week Light + 2 Weeks AMI	$p <$ 1 Week AMI vs 1 Week Lignt + 2 Wks AMI
1	-1.28 ± 0.18	-1.58 ± 0.17	-1.19 ± 0.23	0.02	n.s.	0.02
2	-0.92 ± 0.24	-0.96 ± 0.21	$+0.56 \pm 0.07$	n.s.	0.00005	0.00002
3	-1.31 ± 0.29	-1.59 ± 0.57	$+0.39 \pm 0.07$	n.s.	0.00003	0.002
4	-1.25 ± 0.27	-1.45 ± 0.24	-0.47 ± 0.08	n.s.	0.002	0.0003
5	-1.14 ± 0.30	-1.19 ± 0.27	-0.73 ± 0.07	n.s.	n.s.	0.04
6	-1.61 ± 0.35	-2.96 ± 0.36	-0.63 ± 0.22	0.00004	0.0002	0.000001
7	-1.44 ± 0.19	-1.80 ± 0.17	$+0.44 \pm 0.08$	0.005	0.000001	0.000001
8	-0.52 ± 0.20	-1.90 ± 0.28	-0.09 ± 0.20	0.00004	0.06	0.000004
	-1.19 ± 0.12	-1.68 ± 0.21	-0.22 ± 0.25			

Student's paired t-tests of individual animals hypothermic responses ($n = 8$) to oxotremorine (1.0 mg/kg ip) at baseline (pretreatment), following 7 days of treatment with amitriptyline (10.0 mg/kg ip twice daily), and after 14 days of amitriptyline administration plus 7 days of treatment with 11,500 lux light.

All 8 animals demonstrated significant blunting of the hypothermic response to oxotremorine at $\alpha < 0.04$ after concurrent treatment with AMI and light (sample mean $\pm$ SEM = -0.22 $\pm$ 0.23°C) relative to the AMI phase (-1.68 $\pm$ 0.21°C). The difference in the mean response to oxotremorine under these conditions was highly significant ($p < 0.0015$, $t = 5.39$, $df = 7$).

Bright artificial light was discontinued after two weeks, but treatment with AMI continued for another 7 days. The mean hypothermic response to oxotremorine 7 days after light treatment ceased was -2.03 $\pm$ 0.15°C. This was significantly greater than the mean response during concurrent AMI and light administration ($p < 0.0015$, $t = 5.10$, $df = 7$).

Experiment 3: The mean core temperature of the sample prior to challenge with oxotremorine was 38.0 $\pm$ .3°C. The sample exhibited a hypothermic response to oxotremorine, 1.0 mg/kg ip, of -1.6 $\pm$ .3 at baseline ($p < 0.0001$, $t = 6.34$, $df = 9$). Treatment with AMI produced an increase in hypothermic response of the sample to -2.1 $\pm$ .3 ($p < 0.07$, $t = 2.05$, $df = 9$). The mean hypothermic response to oxotremorine following one week of exposure to 300 lux light was -3.1 $\pm$ .3°C. This differed from baseline ($p > 0.03$, $t = 2.68$, $df = 9$).

Discussion

Chronic treatment with bright artificial light (11,500 lux) blocked the capacity of AMI to produce supersensitivity to the hypothermic effects of oxotremorine. However, treatment with standard fluorescent light at an intensity of 300 lux did not have this effect. This suggests that light intensity is a critical variable. Further, this observation argues against the hypothesis that the effects we measured result from alteration in the circadian rhythms consequent to constant light exposure. The intensity of light in the rats' cages in our vivarium is 300 lux. Sprague-Dawley rats demonstrate clear-cut circadian changes in motor activity and core temperature in response to turning the lights in the vivarium on or off. We therefore propose the results presented in this article are due to the effects of bright artificial light rather than light itself.

Bright light (as opposed to light at an intensity of 300 lux) can have dramatic effects on the amplitude of circadian rhythms. This is certainly true of the circadian temperature rhythm. The simplist means for assessing whether bright artifical light altered the amplitude of the diurnal temperature rhythm is to determine whether mean core temperature measured at the same time of the day, differed prior to and at the conclusion of treatment with bright light. Bright artificial light was not associated with a change in core temperature in either Experiment 1 or 2. This could be the consequence of chance. However, it is possible the cues in the environment entrained the animals so that the amplitude of the circadian temperature rhythm was preserved. For example, human activity around the animals might have served as an external cue allowing them to maintain the typical diurnal temperature amplitude or rhythm.

The hypothermic response to oxotremorine at baseline differ considerably in Experiments 1 (-2.46 $\pm$ 0.41) and 2 (-1.19 $\pm$ 0.12°C). This is partially due to the use of a higher dose of oxotremorine in the first, 1.5 mg/kg ip, as opposed to the second. However, this point aside, one must consider that there is a great deal of variance in the responsiveness of Sprague-Dawley rats to muscarinic agonists (13). This variability of responsivity is simply the consequence of using an outbred line of rats. Secondly, our dose of AMI merits comment since it is, on a milligram per kilogram basis, 2.5-10

times that administered to depressed patients. Doses of antidepressants (e.g., AMI, desipramine, amoxapine and fluoxetine) in the range of 10 mg/kg ip twice daily are commonly used on a chronic basis in preclinical studies using rats without ill effect (11,12,14,15). The animals thrive, gain weight and exhibit no signs of toxicity on these seemingly high doses.

Patients with SAD are typically treated with full-spectrum light at an intensity of 2,500 lux. There are advantages to using higher intensities of bright artificial light in preliminary studies. Our objective was to determine whether bright light, as opposed to standard room lighting, produces subsensitivity to the hypothermic effects of oxotremorine. Use of a high "dose" decreases the probability of accepting a false null hypothesis ("bright artificial light does not produce subsensitivity to the thermic effects of oxotremorine"). Now that it has been demonstrated that treatment with full-spectrum light at an intensity of 11,500 lux results in decreased sensitivity to oxotremorine, and by implication subsensitivity of muscarinic receptors, it would be reasonable to evaluate lower "doses" and the effects of administering light for circumscribed periods of time each day. These studies are now under way.

The light unit we used delivers light at an intensity of 2,500 lux at a distance of 122 cm from a patient's face. However, to deliver light at an intensity of 11,500 lux, the unit was suspended 50 cm above the animals. When placed this distance from the face of a patient, there is no discomfort. Thus, it would be feasible to change the current treatment protocols should studies suggest an intensity of light in excess of 2,500 lux might be superior.

The data presented in this article indicate that bright artificial light (of the variety used to treat SAD) potently produces subsensitivity of a central muscarinic mechanism involved in the regulation of core temperature. Experiment 3 indicates that the findings are not a nonspecific consequence of upsetting circadian rhythms due to constant light exposure. Finally, these results are in complete accord with the cholinergic hypothesis of depression.

Janowsky et al. (16) proposed that depressive disorders are related to a defect in central cholinergic mechanisms. Specifically, the depressed state is characterized by cholinergic overdrive (7-9). Sitaram et al. (14) observed that euthymic affective disorder patients exhibit accelerated onset of REM sleep in response to cholinomimetic challenge relative to normal subjects. This indicates that at least some forms of affective illness involve state independent supersensitivity of a central muscarinic mechanism.

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