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Mammalian cerebral cortical tissue responds to low-intensity visible light

(γ-aminobutyric acid release/neurotransmitter/brain slices/light penetration)

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ABSTRACT **Low levels of visible light directed onto slices of rat cerebral cortical tissue enhanced net potassium-induced release of the neurotransmitter γ-aminobutyric acid (GABA) from these brain slices. At higher light intensity, net potassium-induced release was suppressed. These effects were apparently not from increased temperature. The amount of light enhancing this neurotransmitter release is approximately equal to the amount of light that can penetrate the head and reach the brain at the intensities of sunlight; this was determined by measuring the light entering the rat head through fur, scalp, skull, and dura mater and considering several natural lighting conditions. These results suggest that ambient light may be sufficient to alter the release of transmitters from mammalian cerebral cortex *in vivo*.**

We report the results from a series of experiments that indicate light can cause a response in cerebral cortical tissue. Impetus for the present research was an observation made while studying effects of the food dye Erythrosin B, which was shown to release neurotransmitter from the neuromuscular junction (1, 2) and from rat cerebral cortical tissue (3, 4). In determining whether actions of this photosensitizing (5) dye on brain slices depended on light (4, 6), we noticed that light itself appeared to affect the release of the transmitter. A preliminary report of these studies has been presented (7).

In invertebrates and lower vertebrates, light elicits a variety of responses via photoreceptors located outside the eye. These effects include entrainment of daily hormonal and activity–rest cycles, as well as seasonal reproductive and behavioral rhythms (8). In adult mammals, however, whether light acting at a site other than the eye influences any of these rhythms is unclear. The bulk of current evidence on this point favors light through the eyes as the locus of control. A direct effect of visible light on one of these rhythms, reported to occur via photoreceptors outside the eyes (9), has been attributed by others to a residual effect from the eyes (cf. 10). Although light radiated directly on the hypothalamus can cause a neuroendocrine response in blind adult rats (11), the absence of entrainment of certain rhythms (12, 13) and the absence of electrical responsiveness of hypothalamic cells (14) has led to the suggestion that such direct-light effects found in adult mammalian brain may lack functional significance (12). Even the pineal gland, which in some nonmammalian species appears directly affected by light (13), in mammals is reported to be controlled by light through the eyes (15). Before all photorhythm control in mammals is assumed to be driven by light admitted through the eye, however, Rusak and Zucker (10) have implied that other species or light intensities should be studied.

Precedents exist for light effects on peripheral neural tissue not classically considered as photoreceptive. Laser light in the visible range has been found to affect neural activity in a mammalian nerve–smooth muscle preparation (16), in the abdominal ganglion of the sea slug *Aplysia* (17), and in the human in suppression of clonus when the light is applied to skin (18); the two latter effects occurred without any measured increase in temperature. Laser light that suppresses clonus has been reported to cause a somatosensory evoked potential in human peripheral nerve (19), although we were unable to reproduce this interesting finding (20).

METHODS

Dissection and incubation procedures have been described (21). After decapitation of male rats (Sprague–Dawley) and removal of the brain and of meninges from the brain, ≈0.5-mm thick slices of tissue perpendicular to the radial axis of the brain were removed from as many regions of cerebral cortex as possible. Layers of cortex just beneath the meninges contributed most of the tissue. Slices were placed in Ringer's solution (120 mM NaCl/4 mM KCl/1.8 mM $CaCl_2$/0.8 mM $MgSO_4$/4 mM Na_2HPO_4/15 mM $NaHCO_3$/10 mM glucose, brought to pH 7.35 with HCl and bubbled with 95% O_2/5% CO_2 occasionally) in a Petri dish at room temperature during the 10-min dissection and the 15-min weighing procedure. Slices (totaling ≈45 mg) were then placed on a glass-wool bed in a plastic cone (open at top and bottom) in a 1.5-ml centrifuge tube covered with 1 ml of Ringer's solution. In each experiment, tissue from one brain was randomly distributed into tubes with respect to area of cortex and time after dissection. The slices were allowed to take up γ-amino[^{3}H]butyric acid ([^{3}H]GABA) from the Ringer's solution at 35–36°C in a water bath for 30 min. To prevent GABA metabolism, we included 0.5 mM aminooxyacetic acid in the Ringer's solution (22).

Excess [^{3}H]GABA was removed by rinsing the tissue repeatedly every 3 min and counting the radioactivity in the collected medium until radioactivity reached baseline. Solutions were changed by transferring the cone into a new centrifuge tube and then applying the next rinsing solution. All these steps occurred in daylight plus room fluorescent light. The final 3-min collection period, which provided the baseline neurotransmitter release for each tissue, was in light. Then slices were subjected to Ringer's solution with elevated K^+ (26.4 mM K^+ and reduced NaCl) under either light or dark conditions. The several slices of tissue in each tube were oriented randomly to the light; they partially covered each other, and at least 0.5 cm of Ringer's solution covered the uppermost slice. For the light condition, the tissue was illu-

Abbreviations: GABA, γ-aminobutyric acid; [^{3}H]GABA, γ-amino[^{3}H]butyric acid.

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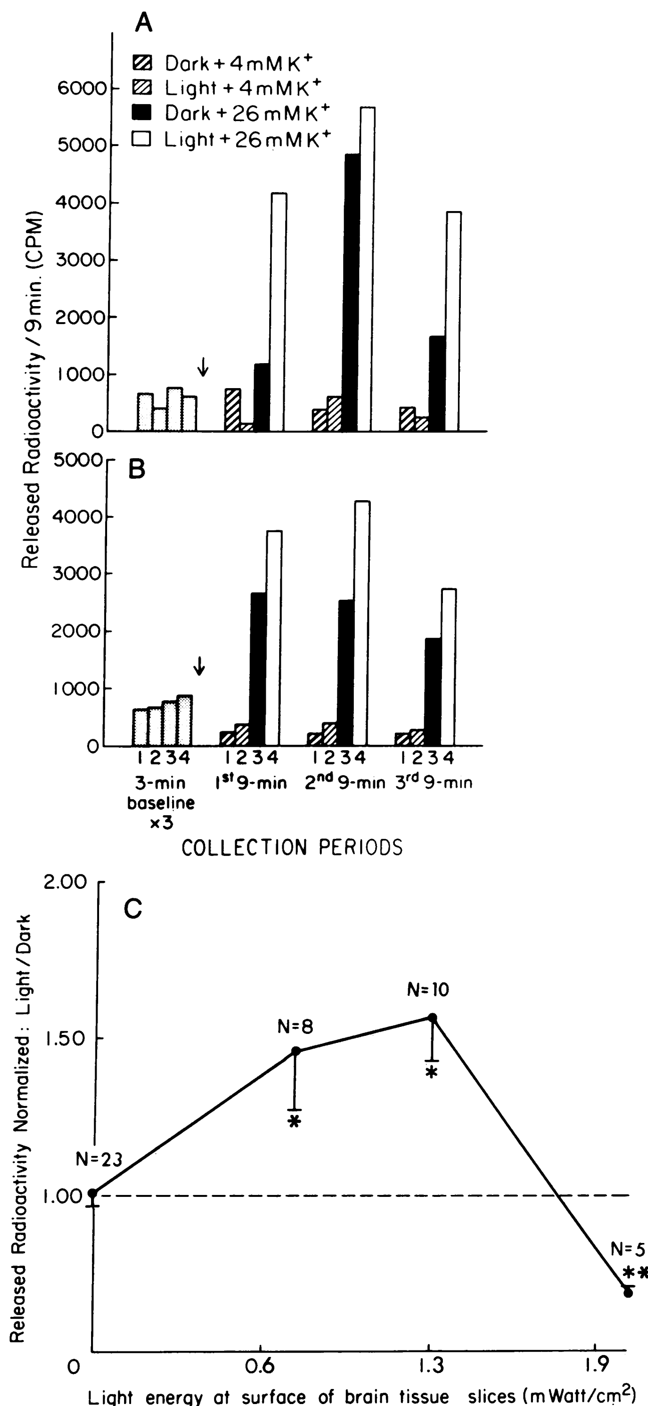

FIG. 1. Response of rat cerebral cortical slices to visible light in the presence of high K^+. Release of radioactivity after incubation of slices with [^{3}H]GABA was measured as described. (*A* and *B*) The slices were rinsed to remove excess radioactivity, and the medium was collected every 3 min in daylight plus room fluorescent light; the last 3-min collection period is shown. Then tissues were subjected (at arrow) to light from a tungsten halogen lamp and high K^+ (26 mM) or to dark and high K^+ for the entire 27 min. Control slices were incubated in normal K^+ (4 mM) in light or dark. The medium was changed and collected every 9 min; "Released Radioactivity/9 min" is that from a 0.1-ml sample from the total 1 ml of medium collected for 9 min. Tubes in conditions 1–4 in the 3-min baseline period correspond to tubes in conditions 1–4 in the three 9-min experimental periods. Values for the 3-min baselines have been multiplied by three to allow a rough comparison with the 9-min experimental periods. Light energy in the 27-min experimental period was ≈1.3 mW/cm^2 at the tissue surface. (*C*) Normalization and compilation of light–dark experiments, including those in (*A* and *B*). Because different pieces of tissue release different baseline amounts of transmitter, each tube with slices provided its own control as follows. Radioactivity in the medium collected from the 27-min exposure to K^+ and to light or dark was summed and divided by radioactivity in the medium from that tissue for the preceding 3 min. For comparison between light and dark, such normalized release of radioactivity in the light condition has been divided by release in the dark. As control for adventitious or systematic differences between release from tissues designated light and ones designated dark, zero-light experiments were conducted (0 light point), in which the geometry of the six tubes of tissue in the water bath and room conditions were as in all other experiments, the only difference being that during the 27-min exposure to high K^+ the tungsten halogen lamp remained off. SEM, *n*, and significant differences from 0-light experiments in Student's *t* test at $P \leq 0.05$ (*) and $P < 0.01$ (**) are indicated.

minated from ≈30 cm above by a tungsten halogen lamp (Sylvania DWY, 650 W, 120 V) in addition to room light, which contributed <1% of the power of the tungsten lamp. To prevent UV transmission, we placed a glass plate between the light source and tissue. Light intensity was measured with a photodiode (diode PV-100 E.G. & G., Salem, MA). For the dark condition, tissue was kept in a darkroom where it was exposed to light (60 W incandescent overhead bulb) for only the 10–20 sec while the medium was changed. Temperature (±0.1°C) was monitored throughout the experiment with the thermometers in centrifuge tubes next to the tissue-containing tubes. Every 9 min (cf. Fig. 1 *A* and *B*) after baseline release was established, the medium was collected for the counting of radioactivity, and fresh medium was then substituted. Ninety-five percent of the released radioactivity can be expected to be in the form of [^{3}H]GABA (23).

Acid-soluble radioactivity remaining in the tissue at the end of an experiment was measured by incubating the tissue with trichloroacetic acid (final concentration, 10%) at 4°C overnight, centrifuging at 13,000 × *g* for 5–10 min, counting the radioactivity in the supernatant, and correcting that count for quenching. This value was then added to the total radioactivity released into the medium during an experiment, thus yielding an estimate of the total radioactivity initially contained in the tissue; the percent radioactivity released was calculated from these values.

Preliminary experiments suggested that the light effect on transmitter release was influenced by several parameters, such as age of the animal, time of day, and season. The early experiments in which these parameters were not controlled yielded inconsistent results. Later experiments, in which these conditions were considered, rendered more consistent results. Therefore, in the particular experiments described here we used young adult (12–23 weeks of age) animals under what seemed to be optimal conditions: midday (between 12:00 and 14:00 or 15:00) and spring and summer seasons. For at least 1 week before the experiment the animals were housed under fluorescent light from 6:30–18:30 standard time. All experiments described in detail in *Results and Discussion* were done according to the above protocol.

Recently, we altered the protocol slightly from that just described—the major difference being that in the present experiments both light and dark conditions were conducted in the same water bath in the darkroom. This last set of experiments will be described only briefly in *Results and Discussion*.

RESULTS AND DISCUSSION

Low-intensity white light (≈1.3 mW/cm^2) enhanced K^+-induced [^{3}H]GABA release, as illustrated by the results of two experiments in Fig. 1 *A* and *B*. As a criterion of tissue responsiveness to light, a light/dark ratio of release was used (see Fig. 1*C* legend for calculation). We present an experi-

ment with a light/dark ratio of 2.56 in which the tissue was most responsive to light (Fig. 1*A*) and another experiment with a ratio of 1.37 in which the tissue was much less responsive to light (Fig. 1*B*). These results were from 2 of the 10 experiments that yielded the 1.3 mW/cm^2 point in Fig. 1*C*. Although the initial observation of a light effect was made with tissue not subjected to high K^+, this light effect was not exhibited by every piece of tissue—e.g., the 4 mM K^+ bars in Fig. 1*A*. To control for differences in uptake by tissues destined for light or dark, we measured radioactivity in the tissue after uptake and rinsing. This initial radioactivity in tissues destined for light divided by the initial radioactivity in tissues destined for dark was 1.05 ± 0.6 (SEM) in eight experiments. From the time a baseline level of released radioactivity was established, the amount of radioactivity released into the medium during the three 9-min incubations with high K^+ and with 1.3 mW/cm^2 light averaged 31 ± 3% (SEM) of the initial tissue radioactivity, based on 15 measurements in five experiments.

By changing white-light intensity we found that the tissue responded with maximum enhancement of [^{3}H]GABA release at ≈1.3 mW/cm^2 (Fig. 1*C*). As the light intensity was increased, tissue response reversed—i.e., inhibition of the K^+-induced release occurred. In addition to the 46 experiments summarized in Fig. 1*C*, similar effects of light were seen in about 30 more experiments under slightly different experimental conditions. For example, older animals required more light for the same effects. Optimally, a set of experiments was done on each group of animals arriving at the same time to determine the best intensity for animals of that age and time of year. Even under apparently optimal conditions, it was not possible to demonstrate a response to light in every animal. For instance, no response (light/dark ratio of 1.09) was seen in one of the 10 experiments at an intensity of 1.3 mW/cm^2.

Using an altered protocol, in which both light and dark conditions occurred in the same water bath, thus insuring similar conditions for the light and dark situation, we obtained qualitatively the same result as in the above experiments. We used this altered protocol to evaluate the effect of light at one low (0.3 mW/cm^2) and at one high intensity (1.9 mW/cm^2) of light. The light-to-dark release ratio (cf. Fig. 1*C* legend for explanation) was greater at the low intensity (1.14 ± 0.07, n = 11) than at the high intensity (0.78 ± 0.04, n = 4). The high intensity was the same intensity that suppressed GABA release in the previous experiments. The difference in release between the low- and high-intensity light conditions was highly significant ($P < 0.005$, Student's t test).

Possible nonspecific means by which light could enhance release, for which we did control experiments, include increased temperature (24), adsorption of GABA to the tissue surface and subsequent release by light or K^+, and increased leakiness of the cell membrane. Increased temperature seems unlikely from the following observations. (*i*) Temperature in the incubation tubes was no higher in the light than in the dark (average variation, light minus dark = −0.1 ± 0.04°C in 119 measurements) in 14 of the experiments of Fig. 1*C*. (*ii*) Because local heating of the tissue from the light could have occurred, a thermister probe (Yellow Springs Instrument model 43TD tele-thermometer) ≈1 mm in diameter, accurate to about 0.1°C, was placed between the uppermost 0.5-mm thick slice of tissue and the several adjacent slices, with at least 1/2 cm of Ringer's solution covering this slice. When the highest intensity of light used in an experiment (that suppressing [^{3}H]GABA release) was directed from above for the entire duration of an experiment (27 min), temperature of the thermister was not increased (Table 1). (*iii*) Only by deliberately increasing the temperature by 1.5°C (from 35–36.5°C) was the increase in release brought into the lower range of that found with light (higher temperature/lower temperature = 1.4). Were heat responsible, raising the temperature a comparable amount to that expected in the light should mimic the response to light. However, given the lack of any demonstrable increase in tissue temperature with light and the large temperature increase needed to increase [^{3}H]GABA release, release by increased temperature and release by light appear to be in different ranges. (*iv*) Wavelengths of light in the infrared region could be heating the tissue directly; however, filters that block infrared light did not eliminate the increased [^{3}H]GABA release. (*v*) If all energy in the same filtered light (≈50 $\mu W/cm^2$ at 540 nM) were converted to heat, temperature in the volume of tissue would not have increased more than ≈0.3°C. [The energy at the surface of the Ringer's solution above the tissue was measured to be 0.05 mW/cm^2. This figure was multiplied by the approximate surface area of the tissue (0.116 cm^2) and by the duration of these experiments (18 min).

Table 1. Measurement of temperature under various light conditions

Light condition	Time after light addition, min	Temperature, °C: Thermister under lighted tissue	Thermometer in water bath ≈4 cm from light
Incandescent overhead bulb (<1% of tungsten halogen lamp)	0	35.7	35.9
	2	35.8	36.0
	4	35.8	35.9
	5	35.8	35.9
	6	35.8	36.0
	8	35.8	35.8
	10	35.7	35.7
	12	35.6	35.6
	13	35.8	35.7
	15	36.0	36.0
	16	36.0	36.0
Tungsten halogen lamp (1.3mW/cm^2)	0	35.8	36.0
	7	35.8	35.8
	14	35.2	35.2
	25	35.9	36.0
	27	35.9	36.1
	30	35.9	36.0
Tungsten halogen lamp (1.9 mW/cm^2)	0	36.0	36.2
	1	36.1	36.2
	6	36.0	36.1
	19	36.0	36.1
	23	36.0	36.2
	26	36.0	36.2

$$\frac{(5 \times 10^{-5}\ \mathrm{W})}{\mathrm{cm}^2} \times \frac{(0.2389\ \mathrm{cal/sec})}{1\mathrm{W}} \times (0.116\ \mathrm{cm}^2) \times 1080\ \mathrm{sec} = 0.0047\ \mathrm{cal},$$

where 1 cal = 4.184 J. Because 1 cal is the energy required to raise 1 cm^3 of water 1°C, the energy converted to heat in the volume of the tissue (≈0.0174 cm^3) would be 0.0047 cal/0.0174 cm^3 = 0.27°C (25).]

This release of GABA into the medium could have been a nonphysiological release not dependent on metabolically active cells, but rather due to adsorption or binding of GABA to the tissue surface and its subsequent release by light and/or K^+. Blocking aerobic metabolism with the respiratory inhibitors (26) 1 mM azide and 10 mM potassium cyanide and then incubating the tissue with [^{3}H]GABA and rinsing as previously released only a small amount of radioactivity into the medium, and light had no effect on the quantity of

radioactivity—experiments indicating that the release depended on metabolically active cells. Finally, to exclude the possibility that light causes a generalized leakiness of the cell membrane, we used a nonfluorescent dye (5, 6-carboxyfluorescein diacetate), which readily traverses the cell membrane, but which once inside the cell is converted to a fluorescent form (M_r 370) unable to cross intact membranes (cf. ref. 27). Although light increased K^+-induced GABA release, it did not release dye. As shown in Table 2, most dye was subsequently found to be intracellular, as indicated by the observation that cell lysis (distilled H_2O) gave a large fluorescent signal. Any increase in fluorescence could have been below the limit of detection; however, the increase in fluorescence upon lysis with distilled water was easily detected. In summary, at least as manifested by a molecule of M_r 370, no leakiness from light was observed.

Experimenter bias was examined by having another person conduct the experimental operations just as described above. All manipulations of the tissues during the period when light was applied and all calculations were done by this "blind" or naive experimenter, who had not been told the expected outcome for any experiment. In six experiments conducted by each individual, no difference was found between the results from the naive experimenter and those from an author (P.D.W.) (Student's t test, $P < 0.01$).

How much light would be expected to reach the brain *in vivo*? We found that the amount of white-light energy in dim-to-bright daylight should be sufficient to penetrate the head of a young adult rat to the cranial cavity and change transmitter release. This estimate of penetration under natural lighting comes from the data in Table 3 and from measurements of sunlight intensities. Clear sunlight between 400- to 800-nm wavelengths at noon in June is calculated to be 55 mW/cm^2, and in overcast daylight is calculated to be 5.5 mW/cm^2 (28, 29). [Of several possible calculations, we used one in which clear incident sunlight at the earth surface was taken to be 100 mW/cm^2 (28) and light between 400–800 nm to be the fraction taken from a graph of incident light vs. wavelength (29).] The measured fraction of incident light penetrating the head, from Table 3 (0.119–0.054), when multiplied by the incident light in each natural condition (55–5.5 mW/cm^2), gives the estimated penetrating intensity (6.54–2.97 mW/cm^2 to 0.654–0.297 mW/cm^2). The extra hemoglobin in the *in vivo* state could be expected to reduce transmitted light. However, extrapolating from the rabbit eye, in which substantial light transmission at different wavelengths occurs, even through its dense circulatory bed (30), leads to the assumption that the vessels of the rat brain would allow as much transmission. Furthermore, our measurements of penetration are not inconsistent with other observations. A small proportion of sunlight can penetrate into the hypothalamus in smaller vertebrates and even in sheep (31). Light penetration to the superficially located cerebral cortex in any animal, including humans (32), would be much greater than to the centrally located hypothalamus.

Table 2. Lack of release of intracellular fluorescent dye from cells during light-induced GABA release

Conditions	Collection periods, min	Fluorescence in medium, arbitrary units		Radioactivity in medium, cpm	
Ringer's solution and	0	22.5	24.5	150	172
room light	3	18.0	17.5	126	121
K^+ plus tungsten		Light	Dark	Light	Dark
lamp or dark	9	18.0	19.0	721	483
	9	18.5	15.0	409	462
	9	13.0	14.0	501	211
Distilled H_2O	9	85.0	83.0	3418	3809

Tissue was incubated with [^{3}H]GABA as described, and with 5,6-carboxyfluorescein diacetate (final concentration, 5 μM) in the Ringer's solution. Rinsing the tissue, applying light or dark plus high K^+, and collecting the medium were done as described in *Methods* and the legend to Fig. 1 *A* and *B*. At completion of the experiment, to determine that dye had been taken up by the cells, we added 1 ml of distilled water to the tissue for 9 min and collected the cell lysate. Radioactivity in the medium was measured as indicated, and fluorescence was read at 520 nM with an excitation wavelength of 492 nM (Perkin–Elmer MPF-44A fluorometer).

Table 3. Measurement of incident light penetrating the unshaven head into the cranial cavity of rats of various ages and weights

Body weight, g	Approximate age, weeks	Incident light penetrating head, %	Intensity of penetrating light at incident light of 9 mW/cm^2,* mW/cm^2
281	11	11.9	1.07
373	18	8.6	0.77
384	15	7.7	0.69
395	16	7.1	0.64
495	16	7.7	0.69
584	23	4.7	0.42
618	21	5.4	0.49

After brains were removed, the photodiode used for measuring intensity was placed in the rat cranial cavity 1 or 2 mm central to the dura mater. Cloths were secured over the neck opening and eyes, the head surface was exposed to white light from the tungsten halogen lamp, and light intensity was measured inside the cavity.

*The value 9 mW/cm^2 lies between the values for overcast daylight and bright sunlight.

Whatever cell types, either neural or glial (33, 34, cf. 35), are releasing GABA, the K^+-induced GABA release presumably occurs due to direct depolarization of the cell membrane. However, we have not ruled out a mechanism of blockade of reuptake. Similarly, we do not know whether the light effect on GABA release is on a stimulation–secretion- coupled mechanism, on a GABA-carrier mechanism, or on some unknown release process. Irrespective of the exact mechanism, this work implies that photoreceptive molecules or processes exist in surface brain tissue and that light might be affecting mammalian brain directly through the head. The first step toward establishing whether a photoreceptive molecule is involved is to determine the wavelengths that support the effect. Determining the generality and relevance of the observed light-mediated response awaits studies on what other transmitters or molecules, what cells, and what *in vivo* processes might be influenced.

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