

EFFECT OF 670-NM LIGHT-EMITTING DIODE LIGHT ON NEURONAL CULTURES

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Introduction

Light close to and within the near infrared range has documented benefits for promoting wound healing in human and animal studies (Conlan et al., 1996; Yu et al., 1997; Sommer et al., 2001). Our preliminary results using light-emitting diodes (LEDs) in this range have also demonstrated two- to five-fold increases in growth-phase-specific DNA synthesis in normal fibroblasts, muscle cells, osteoblasts, and mucosal epithelial cells in tissue cultures (Whelan et al., 2001). However, the mechanisms of action of such light on cells are poorly understood.

Britton Chance's group reported that about 50% of near-infrared light is absorbed by mitochondrial chromophores such as cytochrome c oxidase (Beauvoit et al., 1994), which is the terminal enzyme of the electron transport chain (Wikstrom et al., 1981). Karu's extensive review (1999) indicated that cytochrome oxidase is a key photoacceptor when cells are irradiated with monochromatic red to near-infrared radiation. Cytochrome oxidase is an integral membrane protein and contains four redox active metal centers and has a strong absorbance in the red to near-infrared range detectable in vivo by near-infrared spectroscopy (Cooper and Springett, 1997).

Cytochrome c oxidase (EC 1.9.3.1) is the terminal enzyme of the electron transport system of all eukaryotes, oxidizing its substrate cytochrome c and reducing molecular oxygen to water. It is an important energy-generating enzyme critical for the proper functioning of almost all cells, especially those of highly oxidative organs such as the brain. The level of energy metabolism in neurons is closely coupled to their functional activity, and cytochrome oxidase has proven to be a sensitive and reliable marker of neuronal activity (reviewed in Wong-Riley, 1989).

We hypothesized that the therapeutic effects of such light result from the stimulation of cellular events associated with increases in cytochrome oxidase activity. As a first step in testing our hypothesis, we subjected primary neuronal cultures to impulse blockade by tetrodotoxin (TTX), a voltage-dependent sodium channel blocker, and applied LED light at 670 nm to determine if it could partially or fully reverse the reduction of cytochrome oxidase activity by TTX. The wavelength and parameters were previously tested to be beneficial for wound healing (Whelan et al., 2001).

Methods

Primary neuronal cultures from postnatal day 3-4 rat visual cortex were grown on coverslips inverted onto a glial feeder layer; the two were separated from each other by small wax spheres (Zhang and Wong-Riley, 1999; modification of Goslin and Banker, 1991). The replication of non-neuronal cells was inhibited by cytosine arabinoside, a DNA synthesis blocker. Three culture age groups were used at the start of the experiments: early (5th to 6th day of culture), middle (11th to 12th day), and late (15th to 16th day) groups. The purpose was to determine if the effects observed were age-dependent. Within each age group, cultures were subdivided into a) Control; b) TTX exposure for 6 days; c) TTX exposure for 6 days and LED treatment once per day for the last 5 of the 6 days; and d) LED treatment without TTX, once per day for 5 days. For the early group, we also tested for the effect of one-time LED treatment on the last day of TTX exposure. All experiments were repeated six times.

We used a GaAlAs light emitting diode (LED) array of 670 nm wavelength (bandwidth of 25-30 nm at 50% power), power intensity of 50 mW/cm², and energy density of 4 joules/cm² when applied for 1 minute and 20 seconds. The wavelength and parameters were previously determined to be beneficial for wound healing.

Cytochrome oxidase reactions were performed as described previously (Wong-Riley, 1979; Zhang and Wong-Riley, 1999). Reaction product of cytochrome oxidase activity was measured by optical densitometry via a Zeiss Zonax MPM 03 photometer. Multiple, two-micron spot-size readings were taken from the cytoplasm of each cell. Between 150-300 cells were measured from each group, using a 25x objective lens. The background was subtracted by setting zero over a blank area (without cells) in each slide, and all lighting conditions, magnifications, and reference points were kept constant. Two-tailed Student's t test for paired comparisons and analysis of variance (ANOVA) for group comparisons were used to analyze differences between treated and untreated groups. Results were expressed as mean \pm SEM. A probability of 0.01 or less was considered significant.

Results and Discussion

Results indicated that primary neurons in culture were heterogeneous in size, shapes, and levels of cytochrome oxidase activity. They were classified into three metabolic cell groups: dark, moderate, and lightly reactive for cytochrome oxidase. TTX caused a significant reduction in enzyme levels of all neurons examined, without causing detectable changes in cell size, shape, or viability. This is in agreement with our previous findings (Zhang and Wong-Riley, 1999). In the presence of six days of TTX, 670 nm LED light treatment at 1 min 20 sec per day for five days led to a reversal of the TTX effect, such that cytochrome oxidase levels in all cells reached control levels (Fig. 1). Comparable results were obtained in all three age groups examined: early, middle, and late.

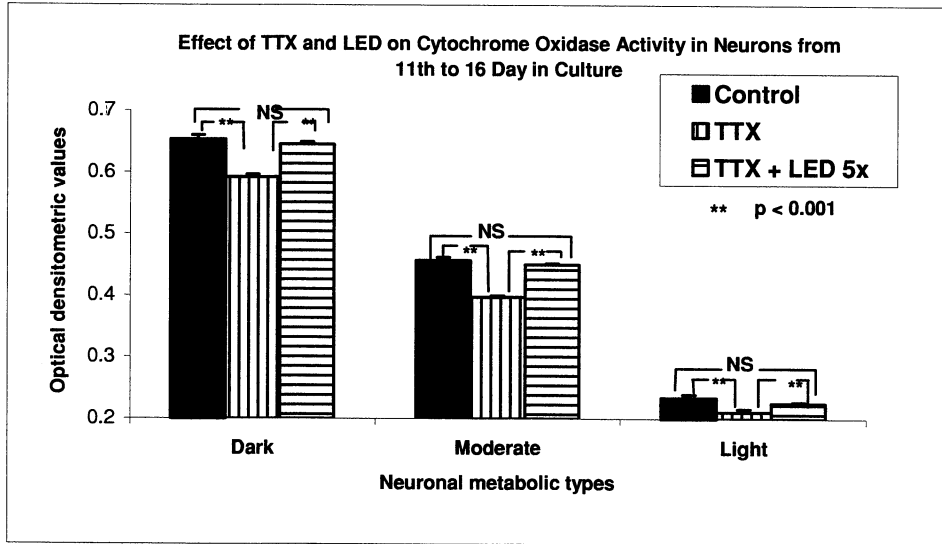


Figure 1

LED treatment alone for five days without the presence of TTX induced an up-regulation of cytochrome oxidase activity that was significantly higher than that of control sister cultures (Fig. 2). To our surprise, a single treatment of LED on control neurons or neurons that had been inactivated by TTX for 5 days brought about a significant increase in cytochrome oxidase activity in darkly reactive neurons, though not in moderately or lightly reactive neurons. This increase, however, did not reach control levels, indicating an incomplete reversal.

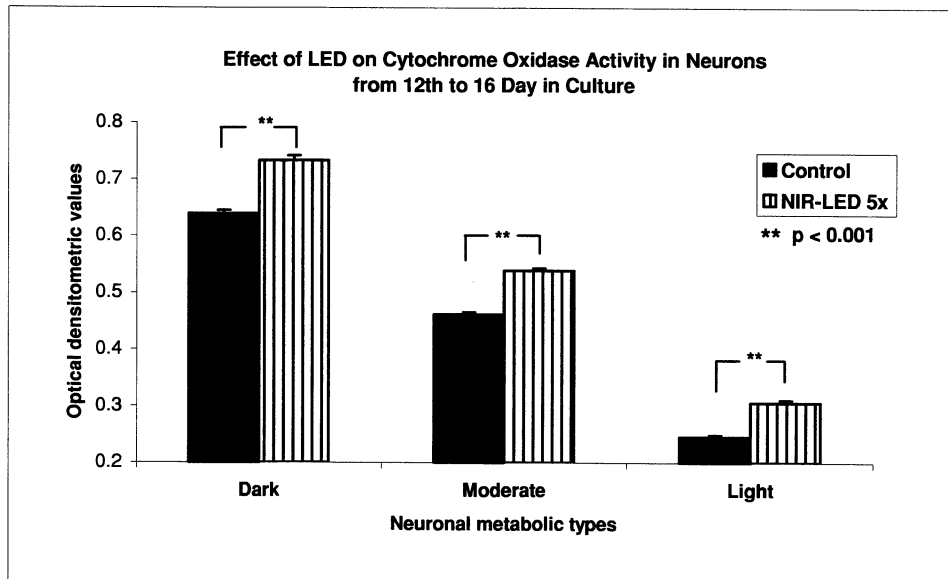


Figure 2

The fact that LED applications of only 1 minute and 20 seconds over a 24 hour period was able to activate and sustain elevated levels of cytochrome oxidase activity during that period indicates that a cascade of events must have been triggered by the initial activation of the enzyme. These events may include the activation of immediate early genes, transcription factors (Zhang and Wong-Riley 2000a), cytochrome oxidase subunit gene expressions (Zhang and Wong-Riley 2000b), subunit protein synthesis, and other metabolic pathways. Future studies will be directed at testing other wavelengths with LED arrays and probing the molecular mechanisms underlying the activation of cytochrome oxidase in neurons by LED.

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