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CNS oxygen toxicity in the rat: role of ambient illumination

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Bitterman N, Melamed Y, Perlman I. CNS oxygen toxicity in the rat: role of ambient illumination. Undersea Biomed Res 1986; 13(1):19–25.—The effect of ambient illumination on sensitivity to CNS oxygen toxicity was studied in awake male rats. Continuous recording of the EEG was obtained with chronically implanted cortical electrodes. The appearance of the electrical discharges in the EEG was used as an end point for CNS oxygen toxicity. It was found that sensitivity to CNS oxygen toxicity was inversely related to the level of ambient light illumination. The latent period for appearance of the electrical discharges was significantly shorter in darkness than in light over most of the oxygen pressures. Rats with severely depressed retinal function were as sensitive to oxygen at high pressure as normal rats in darkness, demonstrating the importance of visual input in the modulation of sensitivity to CNS oxygen toxicity.

hyperbaric oxygen
CNS oxygen toxicity
ambient illumination
rat
EEG

Exposure to high oxygen pressures (HOP) results in clonic-tonic seizures accompanied by electrical discharges in the EEG similar to grand mal epilepsy (1). These electrical discharges can be recorded from a free-moving animal and thus used as an objective index for CNS oxygen toxicity. The sensitivity to CNS oxygen toxicity can be modified by a variety of environmental factors. The latent period for the development of oxygen toxicity changes with the circadian rhythm (2). It is raised in a dry environment as opposed to a wet one (3). Temperature and CO_2 concentration in the breathing gas also affect the sensitivity to oxygen (3, 4). Nagai et al. (5, 6), while reexamining the relationship between brain norepinephrine concentration and HOP-induced seizures in mice, noticed that lighting conditions in the laboratory affected the onset and incidence of seizures during exposure to HOP. Their interesting results were drawn from exposure of mice to a prolonged period of darkness, which could induce major biochemical changes in the brain. Our study was planned to find the effect of the ambient illumination as an environmental factor, which can rapidly

change during the course of a single dive, on CNS oxygen toxicity. It was found that lowering the level of illumination raised the sensitivity of rats to HOP. Experiments performed with retinal-damaged rats supported the hypothesis that the effect of ambient illumination on oxygen toxicity is mediated by the visual system.

METHODS

The experiments were carried out on male rats of Charles River strain weighing 220–280 g. Chronic electrodes were implanted into the skull and attached to a connector fixed with cement to the head. This method allowed continuous recording of the EEG from the awake, unstressed rat moving about freely in the hyperbaric chamber. No postoperative neurological damage was noticed or recorded in the EEG. Up to the day of the experiment, the rats ate commercial food and drank water ad libitum. A few animals were implanted with nickel plates placed on the back, beneath the skin. These were also attached to the connector, thus giving a continuous ECG.

On the day of the experiment, the rat was placed into a 3-liter Plexiglas cage. The small chamber was put into a 150-liter hyperbaric chamber for animal experiments (Roberto-Galleazi, Italy) equipped for recording of physiological parameters. Using the small Plexiglas chamber allowed economical use of oxygen and quick exchange of the breathing gas. While the small chamber was flushed with pure oxygen, the pressure was raised in the big chamber with air. Bubbling of the outgoing oxygen from the small Plexiglas chamber into a water column proved the existence of a small positive pressure preventing any diffusion of air into the oxygen chamber. A constant flow of 2 l/min prevented the accumulation of CO_2 in the chamber.

Light was provided by a 20-W lamp pressed against one of the windows; all other windows were closed. The intensity of the ambient illumination within the chamber was controlled by ''neutral'' density filters placed between the light source and the chamber window. A set of four red LEDs connected to a battery allowed us to watch the bubbling in total darkness. Each rat was kept in the chamber at atmospheric pressure for 15–30 min to adapt it to the experimental conditions.

During the adaptation period, heart rate and EEG were monitored. CNS oxygen toxicity was defined by the time interval between reaching the predetermined experimental pressure and the appearance of the first electrical discharge in the EEG.

Group mean times were tested for significant differences using Student's *t*-test.

RESULTS

A major factor that can contribute to observed changes in sensitivity to oxygen toxicity is stress, which can be induced when ambient illumination is suddenly changed. We therefore observed the rats kept in light or darkness and measured EEG and ECG.

Rats kept in darkness for periods of up to 30 min did not show any significant differences either in behavior or in the electrocorticogram and ECG compared to rats kept in a lighted environment. Electrocardiogram monitoring of the rats breathing atmospheric air in the closed, ventilated, darkened hyperbaric chamber before the beginning of pressurization (up to 30 min) showed no significant change in the animals' heart rates. The electrical discharges appearing on exposure to high oxygen pressures

both in light and in darkness were very similar and have the typical form and severity known for CNS oxygen toxicity.

Two groups of rats were acclimated to darkness for either 15 or 30 min before being exposed to 6 ATA oxygen. No significant difference was found for the latent period for electrical discharges between the two groups of rats, 7.46 min ± 0.47 (SEM) $n = 14$ and 6.22 min ± 0.39 (SEM) $n = 13$, respectively. It was therefore concluded that a period of 15 min was enough to adapt the rats to the lighting conditions and thus to minimize the contribution of stress to the measurements.

Central nervous system oxygen toxicity was measured at different oxygen pressures in rats kept either in total darkness or in bright, ambient illumination. As shown in Fig. 1, the latent period, at every oxygen pressure used, was shorter in total darkness than in light. Below 4.5 ATA our rats did not develop CNS oxygen toxicity in light (at least not before the appearance of pulmonary O_2 toxicity), whereas in darkness the signs of CNS oxygen toxicity could be seen even at 4.2 ATA oxygen.

The inverse relationship between the level of ambient illumination and the sensitivity to oxygen toxicity is further illustrated in Fig. 2. Rats were exposed to 6 ATA oxygen at different levels of light. As the light intensity was reduced, the sensitivity to oxygen toxicity was raised (decrease in latent period) until at dim illumination (2 log units attenuation) a minimum value for the latent period was reached that was not significantly different from the value measured on total darkness.

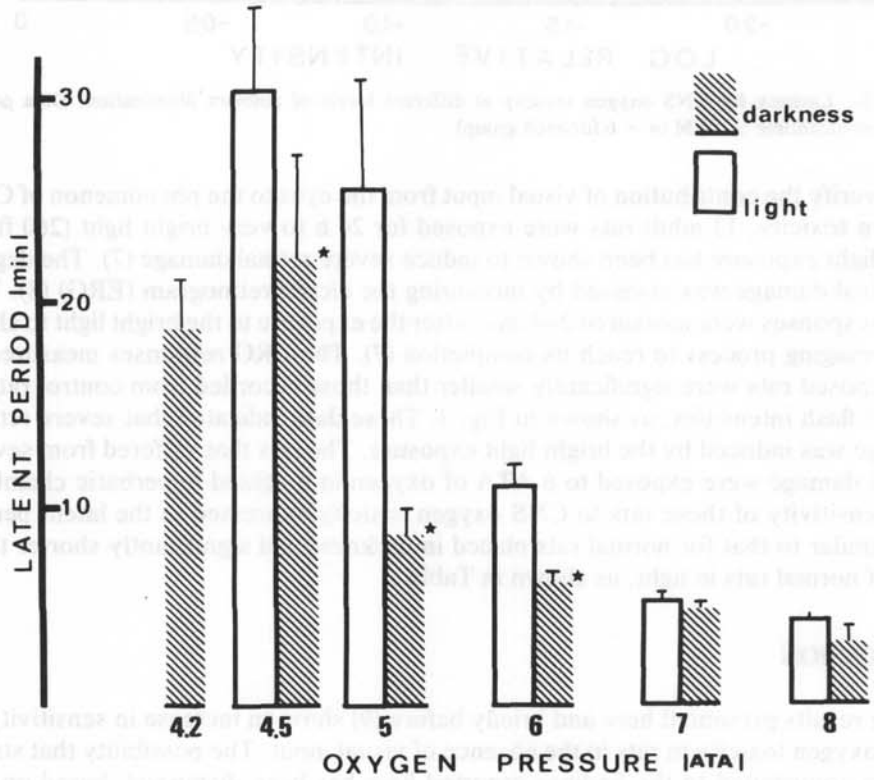

Fig. 1. Latent period at different oxygen pressures studied in unattenuated light and in darkness. Each column represents meantime ± SEM for 6 rats. *Asterisk* indicates significance of $P < 0.001$ in *t*-tests.

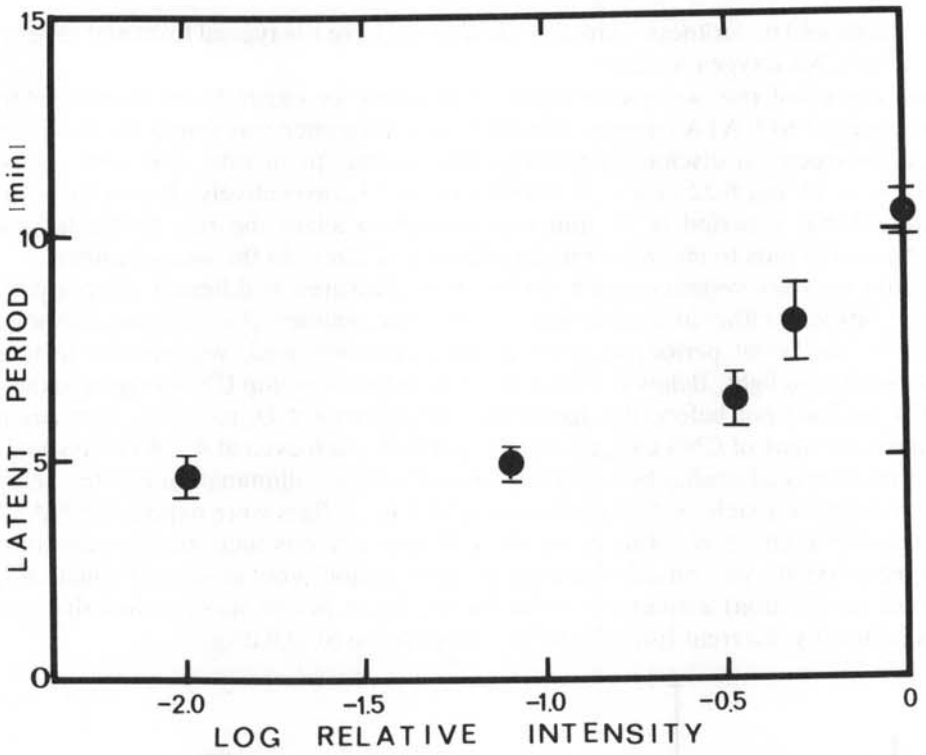

Fig. 2. Latency for CNS oxygen toxicity at different levels of ambient illumination. Data points represent meantime ± SEM (n = 6 for each group).

To verify the contribution of visual input from the eyes to the phenomenon of CNS oxygen toxicity, 13 adult rats were exposed for 24 h to very bright light (260 ft-c). Such light exposure has been shown to induce severe retinal damage (7). The degree of retinal damage was assessed by measuring the electroretinogram (ERG) (8). The ERG responses were measured 2–4 days after the exposure to the bright light to allow the damaging process to reach its completion (7). The ERG responses measured in the exposed rats were significantly smaller than those recorded from control rats at all test flash intensities, as shown in Fig. 3. These data indicated that severe retinal damage was induced by the bright light exposure. The rats that suffered from severe retinal damage were exposed to 6 ATA of oxygen in a lighted hyperbaric chamber. The sensitivity of these rats to CNS oxygen toxicity expressed in the latent period was similar to that for normal rats placed in darkness and significantly shorter than that of normal rats in light, as shown in Table 1.

DISCUSSION

The results presented here and briefly before (9) show an increase in sensitivity to CNS oxygen toxicity in rats in the absence of visual input. The possibility that stress factors contributed to the findings reported here has been dismissed, based on the following: (a) The ECG did not show any change in heart rate, and in the EEG we found no unusual movement artifacts when environmental lighting conditions were

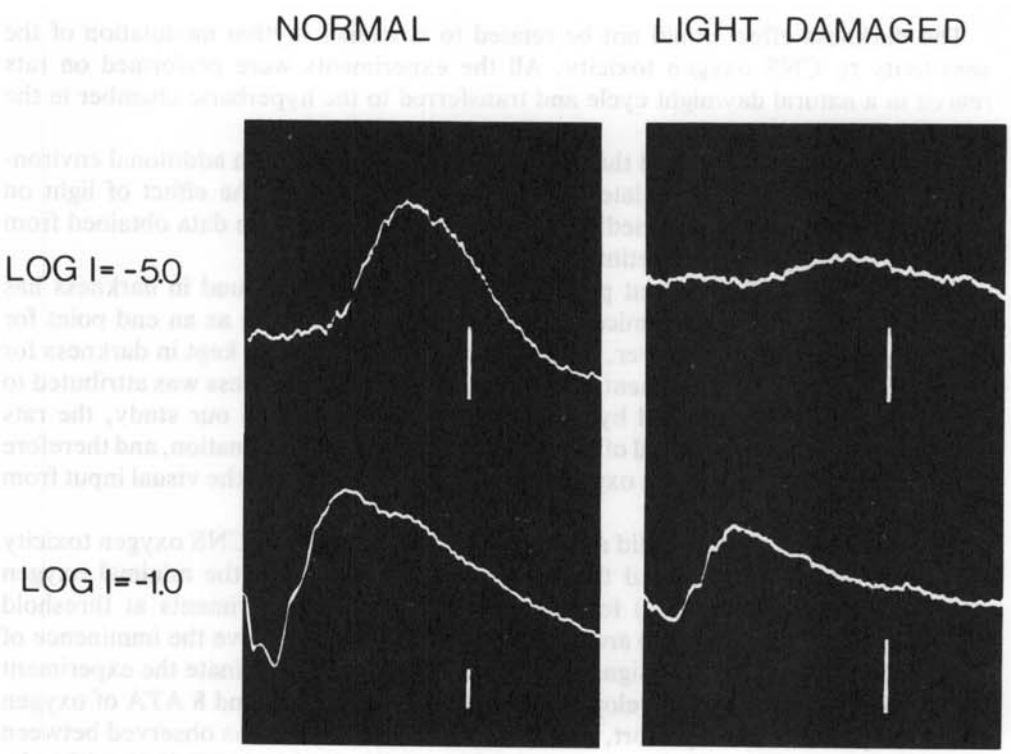

Fig. 3. Electroretinogram responses recorded from normal and light-damaged rat retinae. Flash intensity used to evoke the responses is denoted to the *left* and is expressed by the density of the neutral filter interposed in the light path. Each trace has a length of 180 ms. Calibration *bar* under each trace denotes 200 μV.

TABLE 1

LATENT PERIOD FOR CNS OXYGEN TOXICITY IN DIFFERENT EXPERIMENTAL GROUPS

	Normal		Light Damaged
	Light	Darkness	Light
Latent period (min ± SEM)	10.92 ± 0.8	6.22 ± 0.4	7.15 ± 0.48
n	13	12	12
P (*t*-test)		< 0.001	< 0.001

changed; (b) the rats looked completely relaxed except for the few seconds immediately after the chamber was darkened; and (c) if rats were stressed by darkness, then a decrease in sensitivity to O_2 toxicity (increase in latent period) should have been noticed if the animals were allowed a period of adaptation before oxygen pressure was raised. However, no difference in latent period was observed between rats allowed to adapt to darkness for 15 or 30 min.

The darkness effect could not be related to circadian rhythm modulation of the sensitivity to CNS oxygen toxicity. All the experiments were performed on rats reared in a natural day/night cycle and transferred to the hyperbaric chamber in the morning for experiments.

It is therefore hypothesized that the ambient illumination is an additional environmental factor that can modulate the CNS oxygen toxicity. The effect of light on oxygen toxicity is probably mediated by the visual system. The data obtained from rats with severely damaged retina support this conclusion.

The shortening of the latent period to electrical seizures found in darkness has been previously reported in mice (5, 6), using clinical seizures as an end point for CNS oxygen toxicity. However, in these studies the mice were kept in darkness for about 18 h before the experiment. Therefore, the effect of darkness was attributed to biochemical changes induced by prolonged dark exposure. In our study, the rats were adapted for a short period of time to different levels of illumination, and therefore the observed effect of light on oxygen toxicity was attributed to the visual input from the eye to the brain.

The exposure to darkness did not change the general form of CNS oxygen toxicity in the rat, but only shortened the latent period and lowered the minimal oxygen pressure (threshold pressure) for electrical discharges. Experiments at threshold pressure (4–4.5 ATA oxygen) are complicated, since they involve the imminence of pulmonary oxygen toxicity. Signs of dyspnea caused us to terminate the experiment even before the rat could develop CNS oxygen toxicity. At 7 and 8 ATA of oxygen the latent period was very short, and no statistical difference was observed between rats in light and darkness. There seems to be a minimal critical time interval for the development of CNS oxygen toxicity. This minimal period depends on internal mechanisms and cannot be further reduced by external factors.

A similar pattern for modulation of CNS oxygen toxicity was reported by Bleiberg et al. (10), who showed that high concentrations of CO_2 decreased the oxygen threshold pressure. The latent period was reduced at the median pressure range, with no statistical difference at higher oxygen pressures.

Our results suggest that it is the visual input from the eyes which modulates CNS oxygen toxicity. However, understanding the mechanisms of this effect needs more work. Is there a nonspecific mechanism common to all sensory modalities that influences the sensitivity of the CNS to HOP, or does each sensory system operate independently via a separate mechanism?

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Bitterman N, Melamed Y, Perlman I. Toxicité à l'oxygène du CNS chez le rat: rôle de l'illumination ambiante. Undersea Biomed Res 1986; 13(1):19–25.—L'effet de l'illumination ambiante sur la sensibilité du CNS à la toxicité à l'oxygène fut étudié chez des rats mâles éveillés. L'enregistrement continu de EEG fut obtenu à l'aide d'électrodes corticales implantées chroniquement. L'apparition de décharges électriques dans l'EEG fut utilisée comme point final pour la toxicité à l'oxygène du CNS. Il fut trouvé que la sensibilité du CNS à la toxicité à l'oxygène était inversement reliée au niveau d'illumination de la limière ambiante. La période de latence pour l'apparition des décharges électriques était significativement plus courte dans la noirceur que dans la lumière pour la plupart des pressions d'oxygène. Les rats avec une fonction rétinienne sévèrement déprimée étaient aussi sensible à l'oxygène à haute

pression que les rats normaux à la noirceur, démontrant ainsi l'importance des stimuli visuels dans la modulation de la sensibilité du CNS à la toxicité à l'oxygène.

oxygène hyperbare
toxicité à l'oxygène du CNS
illumination ambiante
rat
EEG

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