

## LOW FREQUENCY ELECTROMAGNETIC FIELD INFLUENCE ON BRAIN REDOX BALANCE IN THE RAT

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**Abstract.** The study investigated the effect of light – dark cycle (L:D) alteration on the redox balance in different segments of the white Wistar rat brain. Several animal groups were entrained to 24, 48, 72 hour and 7 day continuous light (L:L), and to 24, 48, 72 hour and 7 day continuous dark (D:D), respectively. Artificial electric light intensity attained  $180 \pm 20$  lx, corresponding to low frequency electromagnetic fields (EMF) of about 60 Hz. Malondialdehyde (thiobarbituric acid reactive species test), superoxide dismutase (Minami method), catalase (Aeby technique modified by Beers and Sizer), glutathione peroxidase (Gross and Buetler method) and reduced glutathione (Buetler technique) were assayed in diencephalon, brain stem and cortex. A differentiated stimulation of monitored oxidative and reducing factors occurred in accordance with L:L or D:D synchronization, its duration and the brain structure focused upon. The results were considered to express the photoneuroimmunoendocrine axis activation equally due to endogenous and exogenous EMF interactions, able to interfere with redox electrochemical processes. A quantal cell clock model is suggested, based on possible estimations of the redox enzymatic system dynamic evolution involving reactive oxygen species generation and unpaired electrons scavenging rate.

**Keywords:** nyctohemerality, oxidative stress, bioelectromagnetism, radical cell clock

**Rezumat.** Studiul de față a investigat efectul modificării ciclului lumină – întuneric (L:D) asupra echilibrului redox în diverse segmente ale creierului la șobolani albi Wistar. Grupurile de animale au fost sincronizate la 24, 48, 72 de ore și 7 zile lumină continuă (L:L) și, respectiv, la 24, 48, 72 de ore și 7 zile întuneric continuu (D:D). Intensitatea luminii electrice artificiale a atins  $180 \pm 20$  lucși, corespunzând unor câmpuri electromagnetice de frecvență joasă (CEM), de aproximativ 60 Hz.

S-au evaluat la nivelul diencefalului, trunchiului cerebral și scoarței: malondialdehida (MDA – testul speciilor reactive cu acid tiobarbituric), superoxid dismutaza (SOD – metoda Minami), catalaza (CAT – tehnica Aeby modificată de Beers și Sizer), glutation peroxidaza (GPX – metoda Gross și Buetler) și glutationul redus (GSH-tehnica Buetler). S-a constatat producerea unei stimulări diferențiate a factorilor oxidativi și reducători monitorizați, în concordanță cu sincronizarea la L:L sau D:D, cu durata acesteia și cu structura cerebrală analizată. S-a considerat că rezultatele exprimă o activare a axei fotoneuroimmunoendocrine, eveniment datorat deopotrivă interacțiunii CEM endogene și exogene, capabile să interfereze cu procesele electrochimice redox. Se sugerează posibilitatea conceperii unui nou model cuantic de ceas celular radicalar bazat pe estimarea evoluției dinamice a sistemelor enzimatiche oxidoreducătoare, raportând viteza de generare a speciilor reactive de oxigen la aceea de neutralizare a electronilor nepereche.

**Cuvinte cheie:** ciclu nictemeral, stress oxidativ, bioelectromagnetism, ceas celular radicalar

## INTRODUCTION

**Aim of the study.** This study investigated the effect of light – dark cycle modification on oxygen free radical levels in different segments of the rat brain assaying the extent of both oxidative stress (MDA) and antioxidative response expressed by enzymatic (SOD, CAT, GPX) and nonenzymatic parameters (GSH). Since both natural and artificial (electric) light used in this experiment constitutes an oscillatory exposure to electromagnetic fields (EMFs) the results were also interpreted in terms of electromagnetic field (EMF) interference.

**State of the art.** Analyses dedicated to redox balance biorhythms are rather scarce in mammals. Most of them illustrate the chronogenetic cell clock model, focusing especially on different biosynthesis rates (glutathione redox cycle, cytochrome P-450 isoenzymes, melatonin, NO etc.). More frequently such investigations evaluated redox enzyme circadian rhythms either in plants or in bacteria (1÷14). As expected, in all these cases, oxidative processes and part of the antioxidative defense system activities prevail during the daytime, while reducing phenomena predominate at night. Light, a principal electromagnetic exogenous zeitgeber, represents, indeed, a prooxidative agent as well.

Oxidative and antioxidative indices were shown to describe not only circadian (CD) variations, but circannual (CA) ones, too (2,15). Such oscillations correlate with periodical changes in light intensity and duration, O<sub>2</sub> pressure, temperature, quality and

quantity of food intake, energy metabolism.

**Rationale.** Our experiments investigated the central nervous system (CNS), since the nervous tissue has proved to be extremely susceptible to oxidative damage and also because chronobiologically it stands for the temporal behavioral integration system of the organism. In fact, the hypothalamic suprachiasmatic nuclei are considered the biologic clock in mammals. They are connected to the pineal gland, to its periodical melatonin synthesis and release, but equally to the retina, to its rhythmic neuro-humoral light signalling phenomena. Besides, light exposure corresponded to an EMF influence periodically oscillating over the 24 h span in its intensity and wave length, according to season and time of day.

The research we have undertaken tried to outline the evolution of several redox parameters following natural light – dark cycle alteration, the more so as free radicals generated during oxidative phenomena interfere practically with all cell clock gears envisaged up to now when they do not give rise to a special timekeeping device themselves (16,17).

## MATERIALS AND METHODS

Age matched adult (8 months old) male Wistar rats weighing about 150 ± 30 g were used. Food and water were available *ad libitum*. Different groups of rats were synchronized to 24, 48, 72 h and one week continuous light (L:L) and continuous dark (D:D) respectively. Controls (L:D) were kept on a natural lighting schedule. Each experimental group comprised 6 animals and were

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synchronized to 12:12 (L:D) for fourteen days before exposure to modified L:D cycles. Light intensity was  $180 \pm 20$  lx. Room temperature was  $20 \pm 2^\circ\text{C}$ . Animals were sacrificed by decapitation each time at 08.30 am. Measurements were carried out on rat diencephalon, brain stem and cerebral cortex homogenates. The samples were diluted 1:10 (w/v) in NaCl 0.154 M and centrifuged at 2500 g in order to separate the cellular detrituses. The supernatant was removed and used for the assessments. All operations were performed at  $0^\circ\text{-}4^\circ\text{C}$ , the low temperature counteracting enzyme and GSH impairment.

**MDA** (malondialdehyde), a hallmark of lipid peroxidation, was assessed according to Buege and Aust method (TBARS— Thiobarbituric acid reactive species test) and proteins were determined by Lowry technique (18). MDA levels were expressed in nmol per mg protein (19).

**GSH** (reduced glutathione), a nonenzymatic chain breaking antioxidant, owes its reducing capacity to the presence of highly reactive SH groups. GSH was estimated according to Buetler method (25,28). GSH reduces 5,5-dithio-bis-nitrobenzoic acid 0.01 M to 2-nitrothiobenzoic acid, an adduct that absorbs light at  $\lambda = 412$  nm. The amount of GSH was evaluated by plotting the results against a standard curve calculated for GSH standards. GSH was expressed in  $\mu\text{g}/\text{mg}$  protein.

**NO** (nitrogen monoxide) behaves as both a free radical and a potential free radical scavenger, depending on its concentration and biochemical milieu

(29,30). It was determined by HPLC and expressed percentually as a fluorescence degree (Fig. 1a,b,c,d).

**SOD** (superoxide dismutase, -[EC-1.15.1.1]-), an ubiquitous enzyme, catalyzes  $\text{O}_2^-$  dismutation to  $\text{H}_2\text{O}_2$  and  $\text{O}_2$ . SOD was determined by Minami method, consisting in nitroblue tetrazolium (NBT) reduction to blue formazan by  $\text{O}_2^-$ . The enzymatic activity was given in U/mg prot. (20).

**CAT** (catalase-[EC-1.11.1.6]-), detected particularly in mitochondria and peroxisomes but, to a lower extent, also extracellularly, dismutates  $\text{H}_2\text{O}_2$ . CAT was evaluated using Aeby method modified by Beers and Sizer (21) and was expressed in U/mg prot. Hydrogen peroxide breakdown was measured spectrophotometrically at 240 nm and expressed in U/mg prot.

**GPX** and **GSH** are components of GSH redox cycle (22).

**GPX** (glutathione peroxidase-[EC-1.11.1.9]-), a GSH coenzyme probably equally stimulated by melatonin (23), participates as a catalyst in lipoperoxidation chain blocking reactions. This selenium-dependent GPX activity was assayed following Gross and Buetler procedure based on a spectrophotometric evaluation of glutathione oxidation (24÷26). GPX activity was expressed in  $\mu\text{g}/\text{min}/\text{mg}$  prot.

SOD, CAT and GPX are considered preventive enzymatic antioxidants, since they oppose radical chain reaction initiation.

For all these assessments, standard deviation and statistical significance (t-Student test) were calculated.

RESULTS

Tables 1÷5 indicate the values of MDA, SOD, CAT, GPX and GSH, in normal and experimental conditions.

**MDA** (table 1) increased in almost all tissue samples belonging to animals kept either in 24 h continuous light (355.67 nmol/mg prot.) or 24 h continuous dark (244.17 nmol/mg prot.). Differences existed between the various brain segments investigated. Lipid peroxidation was markedly higher in the cortex and diencephalon in constant dark while in the diencephalon it appeared to be higher in constant light. MDA increased less in the brain stem and cortex under constant light. After seven day continuous light (179 nmol/mg prot. cortex – 255 nmol/mg prot. stem – 200 nmol/mg prot. diencephalon) and seven day continuous dark (18.9 nmol/mg prot. cortex – 196.5 nmol/mg prot. brain stem – 537 nmol/mg prot. diencephalon) exposure MDA raised again to higher values than those attained at 72 h. MDA diminished in the animals synchronized to 48 h continuous light and continuous dark respectively. Its decrease was more important in the brain stem samples. In 72 h entrained animals either to constant light or constant dark, MDA maintained lower levels but, nevertheless higher than normal values.

**SOD** (table 2) remained within the normal range or described reduced levels at 24 h in both constant light and constant dark synchronized laboratory animals. However, SOD decline was much more important in samples from rat brains entrained to 24 h constant dark. SOD concentration was the lowest in the brain stem (0.32

U/mg prot.) and cortex (0.1 U/mg prot.). For a 48 h continuous light entrainment SOD reached peak values in all the brain segments examined and then decreased slightly in the 72 h continuous light synchronized samples, without always coming back to normal concentrations. In rat brain samples from animals synchronized to 48 h constant dark, a rather weak rise was observed while at 72 h DD the enzyme began to decline approaching control levels.

**CAT** (table 3) displayed the lowest concentrations at 24 h in the cortex, both in continuous light (2.95 U/mg prot.) and continuous dark (3 U/mg prot.) synchronized animals. For the same time span synchronization it showed instead increased values in the brain stem (LL:7.77, DD:4.93) and diencephalon (LL:8.19, DD:3.42). For the 48 h entrained animals CAT raised in the cortex in continuous dark to 5.22 and to only 3.1 in continuous light. In brain stem samples the enzyme decreased to 4.27 in DD and to 4.32 in LL while in the diencephalon it peaked most intensely up to 8.41 in LL and to 9.41 in DD. In rats kept on a 72 h constant dark schedule CAT reached very elevated concentrations in the brain stem (15.42), while in the same segment in constant light it raised to only 5.38. In the diencephalon probes CAT came to 8.83 U/mg prot. in DD but diminished to 3.8 U/mg prot. in LL. In the cortex the 72 h constant dark programme determined a huge rise from 5.22 to 13.19 U/mg prot. and a slight one from 3.1 to 5.1 U/mg prot. in LL.

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**Table 1. Comparison between MDA values under normal and experimental conditions (D:D and L:L)**

| MDA        | CORTEX              |        | BRAIN STEM          |        | DIENCEPHALON        |        |
|------------|---------------------|--------|---------------------|--------|---------------------|--------|
|            | mean                | SD     | mean                | SD     | mean                | SD     |
| Control    | 99.58               | 4.518  | 158.07              | 3.532  | 101.33              | 3.670  |
| D:D 24 h   | 248.07 <sup>a</sup> | 13.188 | 285.88 <sup>a</sup> | 15.124 | 382.42 <sup>a</sup> | 12.043 |
| D:D 48 h   | 272.08 <sup>a</sup> | 14.934 | 266.07 <sup>a</sup> | 8.116  | 260.83 <sup>a</sup> | 10.393 |
| D:D 72 h   | 162.06 <sup>a</sup> | 9.970  | 106.36 <sup>a</sup> | 8.705  | 166.37 <sup>a</sup> | 4.931  |
| D:D 7 days | 189.33 <sup>a</sup> | 24.150 | 196.50 <sup>a</sup> | 10.687 | 520.00 <sup>a</sup> | 11.171 |
| L:L 24 h   | 355.70 <sup>a</sup> | 12.318 | 163.38 <sup>a</sup> | 19.093 | 253.48 <sup>a</sup> | 10.976 |
| L:L 48 h   | 152.88 <sup>a</sup> | 7.133  | 140.33 <sup>a</sup> | 8.330  | 200.60 <sup>a</sup> | 19.036 |
| L:L 72 h   | 121.60 <sup>a</sup> | 11.224 | 186.60 <sup>a</sup> | 3.733  | 188.62 <sup>a</sup> | 8.652  |
| L:L 7 days | 179.00 <sup>a</sup> | 10.401 | 255.00 <sup>a</sup> | 16.413 | 203.95 <sup>a</sup> | 10.697 |

a)  $p \leq 0.001$

**Table 2. Comparison between SOD values under normal and experimental conditions (D:D and L:L)**

| SOD        | CORTEX            |       | BRAIN STEM        |       | DIENCEPHALON      |       |
|------------|-------------------|-------|-------------------|-------|-------------------|-------|
|            | mean              | SD    | Mean              | SD    | mean              | SD    |
| Control    | 0.93              | 0.166 | 0.72              | 0.211 | 0.66              | 0.115 |
| D:D 24 h   | 0.55 <sup>a</sup> | 0.128 | 0.12 <sup>a</sup> | 0.081 | 0.57              | 0.308 |
| D:D 48 h   | 1.01              | 0.467 | 0.83 <sup>a</sup> | 0.454 | 1.02              | 0.496 |
| D:D 72 h   | 0.96              | 0.104 | 1.35 <sup>a</sup> | 0.033 | 0.96 <sup>b</sup> | 0.102 |
| D:D 7 days | 0.95              | 0.244 | 0.73 <sup>a</sup> | 0.224 | 0.63              | 0.160 |
| L:L 24 h   | 0.66 <sup>b</sup> | 0.183 | 0.88              | 0.110 | 0.90 <sup>b</sup> | 0.108 |
| L:L 48 h   | 2.83 <sup>a</sup> | 0.492 | 2.42 <sup>a</sup> | 0.455 | 2.63 <sup>b</sup> | 0.481 |
| L:L 72 h   | 1.31 <sup>a</sup> | 0.175 | 1.72 <sup>a</sup> | 0.057 | 2.25 <sup>b</sup> | 0.049 |
| L:L 7 days | 0.88              | 0.186 | 0.54              | 0.261 | 1.03 <sup>b</sup> | 0.152 |

a)  $0.001 \leq p \leq 0.003$

b)  $0.01 \leq p \leq 0.03$

**Tab. 3. Comparison between CAT values under normal and experimental conditions (D:D and L:L)**

| CAT        | CORTEX             |       | BRAIN STEM         |       | DIENCEPHALON      |       |
|------------|--------------------|-------|--------------------|-------|-------------------|-------|
|            | mean               | SD    | mean               | SD    | mean              | SD    |
| Control    | 3.20               | 0.231 | 1.41               | 0.223 | 2.65              | 0.235 |
| D:D 24 h   | 2.76               | 0.414 | 4.88 <sup>b</sup>  | 0.561 | 3.37 <sup>c</sup> | 0.563 |
| D:D 48 h   | 6.28 <sup>a</sup>  | 1.424 | 4.61 <sup>b</sup>  | 0.780 | 8.40 <sup>a</sup> | 0.801 |
| D:D 72 h   | 13.04 <sup>a</sup> | 0.559 | 15.30 <sup>b</sup> | 0.817 | 8.68 <sup>a</sup> | 2.321 |
| D:D 7 days | 4.41 <sup>a</sup>  | 0.769 | 4.04 <sup>b</sup>  | 0.334 | 1.05 <sup>a</sup> | 0.325 |
| L:L 24 h   | 3.11               | 0.644 | 7.72 <sup>b</sup>  | 0.535 | 7.80 <sup>a</sup> | 0.419 |
| L:L 48 h   | 3.18               | 0.780 | 5.13 <sup>b</sup>  | 0.778 | 9.41 <sup>a</sup> | 0.777 |
| L:L 72 h   | 4.95 <sup>a</sup>  | 0.774 | 5.23 <sup>b</sup>  | 0.476 | 3.65 <sup>a</sup> | 0.466 |
| L:L 7 days | 3.58               | 0.374 | 5.78 <sup>b</sup>  | 0.512 | 2.15 <sup>c</sup> | 0.327 |

a)  $p < 0.001$

b)  $p < 0.0001$

c)  $p \leq 0.01$

**GPX** (table 4) was higher in rat brain samples from animals kept in 24 h continuous light and then decreased to normal or even lower values at 48 h and 72 h (continuous light). In constant dark GPX proved to decline at 24 h in the cortex (8.9) and then raised markedly in 48 h entrained animals. The rises were more prominent in the brain stem (32.2  $\mu\text{g}/\text{min}/\text{mg}$  prot.) and especially in the diencephalon (43.9  $\mu\text{g}/\text{min}/\text{mg}$  prot.) equally under 48 h constant darkness. GPX levels diminished in the brain samples from rats kept in 72 h constant dark. GPX intense activity under D:D supports the observation that it could be stimulated by melatonin (23).

GSH (table 5), a nonenzymatic parameter, showed highly elevated levels at 24 h continuous light in all cerebral tissue samples. In the brain

stem it reached 233.4  $\mu\text{g}/\text{mg}$  prot., while in the diencephalon it attained 183.4  $\mu\text{g}/\text{mg}$  prot. and only 153.36  $\mu\text{g}/\text{mg}$  prot. in the cortex. The reducing agent dropped in 48 h continuous light exposed animals and slightly diminished again at 72 h in LL. In 24 h constant dark synchronized animals GSH decreased. It continued to lower at 48 h, and then began to raise to different levels in the different CNS fragments investigated: 88.16  $\mu\text{g}/\text{mg}$  prot. in the diencephalon (D:D); 129.4  $\mu\text{g}/\text{mg}$  prot. in the cortex (D:D) and 135.26  $\mu\text{g}/\text{mg}$  prot. in the brain stem. It is well known that GSH exhibits a circadian rhythm in rats marking its peak levels in the morning (09.00 am) and trough concentrations at night (09.00 pm) (31).

**Table 4. Comparison between GPX values under normal and experimental conditions (D:D and L:L)**

| GPX        | CORTEX             |       | BRAIN STEM         |       | DIENCEPHALON       |       |
|------------|--------------------|-------|--------------------|-------|--------------------|-------|
|            | mean               | SD    | mean               | SD    | mean               | SD    |
| Control    | 16.71              | 0.940 | 17.76              | 0.852 | 12.67              | 0.685 |
| D:D 24 h   | 8.75 <sup>a</sup>  | 0.458 | 17.56              | 0.480 | 11.35 <sup>b</sup> | 0.645 |
| D:D 48 h   | 13.43 <sup>a</sup> | 2.081 | 33.32 <sup>a</sup> | 1.468 | 43.92 <sup>b</sup> | 2.167 |
| D:D 72 h   | 24.23 <sup>a</sup> | 1.406 | 14.38 <sup>a</sup> | 1.073 | 15.19              | 1.898 |
| D:D 7 days | 17.93              | 1.069 | 19.56 <sup>a</sup> | 0.780 | 18.12 <sup>b</sup> | 0.653 |
| L:L 24 h   | 51.95 <sup>a</sup> | 3.370 | 55.75 <sup>a</sup> | 3.569 | 25.12 <sup>b</sup> | 0.468 |
| L:L 48 h   | 23.40 <sup>a</sup> | 1.547 | 23.00 <sup>a</sup> | 2.045 | 20.90 <sup>b</sup> | 1.930 |
| L:L 72 h   | 12.73 <sup>a</sup> | 1.150 | 21.11 <sup>a</sup> | 2.305 | 11.56              | 0.965 |
| L:L 7 days | 17.96              | 1.069 | 19.48 <sup>a</sup> | 0.844 | 10.75 <sup>b</sup> | 0.647 |

a)  $p \leq 0.005$

b)  $p < 0.006$

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**Table 5. Comparison between GSH values under normal and experimental conditions (D:D and L:L)**

| GSH        | CORTEX              |       | BRAIN STEM          |       | DIENCEPHALON        |       |
|------------|---------------------|-------|---------------------|-------|---------------------|-------|
|            | Mean                | SD    | mean                | SD    | mean                | SD    |
| Control    | 55.30               | 0.740 | 57.30               | 0.990 | 70.23               | 0.984 |
| D:D 24 h   | 78.65 <sup>a</sup>  | 3.255 | 83.08 <sup>b</sup>  | 1.146 | 90.78 <sup>c</sup>  | 3.024 |
| D:D 48 h   | 64.93 <sup>a</sup>  | 2.362 | 35.93 <sup>b</sup>  | 1.445 | 33.36 <sup>c</sup>  | 1.765 |
| D:D 72 h   | 129.40 <sup>a</sup> | 1.109 | 135.25 <sup>b</sup> | 6.187 | 88.16 <sup>c</sup>  | 1.515 |
| D:D 7 days | 51.00 <sup>a</sup>  | 2.565 | 48.56 <sup>b</sup>  | 2.753 | 53.26 <sup>c</sup>  | 1.852 |
| L:L 24 h   | 153.26 <sup>a</sup> | 2.580 | 233.35 <sup>b</sup> | 1.597 | 183.31 <sup>c</sup> | 5.161 |
| L:L 48 h   | 90.46 <sup>a</sup>  | 4.427 | 65.68 <sup>b</sup>  | 1.636 | 66.94 <sup>c</sup>  | 1.379 |
| L:L 72 h   | 52.40 <sup>a</sup>  | 1.771 | 53.36 <sup>b</sup>  | 2.736 | 57.96 <sup>c</sup>  | 1.420 |
| L:L 7 days | 55.06               | 1.771 | 65.36 <sup>b</sup>  | 1.708 | 58.42 <sup>c</sup>  | 1.831 |

a)  $p \leq 0.004$

b)  $p \leq 0.007$

c)  $p \leq 0.0007$

NO (Fig.1), another nonenzymatic variable, assessed only in rats entrained to 24 h D:D, displayed higher values in all CNS segments which were focused upon. Its level was particularly increased in the brain stem and diencephalon, supporting these regions contribution to biorhythmic behaviour control (31).

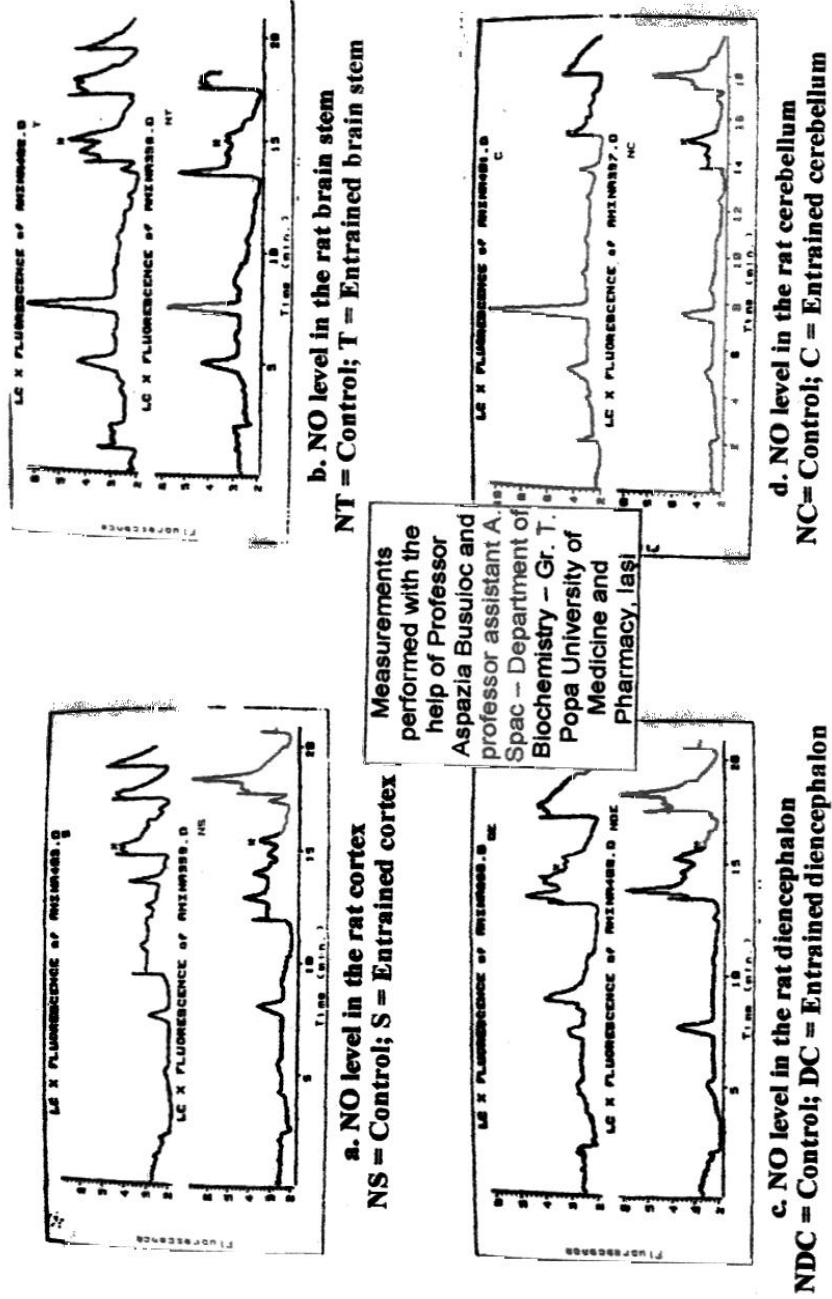
**DISCUSSION**

The experiment used room light intensity ( $\cong 60$  Hz:180±20 lux) mimicking circumstances of common environmental illumination stress. Rats reacted to light – cycle alteration, which represented both an electromagnetic synchronizer and a stressor for their CNS. This more or less specific impact resulted in the appearance of an oxidative stress expressed by modified values of the redox indices that have been analyzed. The antioxidative enzymatic defense systems proved to be differentially affected—induced, inhibited or activated—under the experimental circumstances,

suggesting that they develop complementary oscillatory functions in the biological time and space. Exposure to L:L/D:D presumably modulated a self-selected free-running CD (L:D) cycle.

Light caused a general increase in SOD, CAT, GPX and GSH to a different extent according to the brain region and the illumination period. Darkness instead was characterized by lower SOD, CAT and GSH levels while MDA was relatively higher also depending on the brain segment and dark exposure duration. Usually SOD activity increased in most regions of the stressed brain. In our study the highest values were observed at 48 h under LL. This is in good keeping with the hypothesis of Escobar et al. (32), who considers that an elevated free radical and particularly  $O_2$  quantity inhibits SOD increase in the first stages, favoring the autocatalysis of the enzymatic reaction (33,34).

**Fig. 1 - NO level in the rat brain cortex, diencephalon and cerebellum assessed by HPLC after 24 h D:D synchronization**



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SOD can be inhibited by high NO concentrations, too. NO binds to the active center of the enzyme. Another set of experiments we undertook recently showed, indeed, elevation of NO levels (HPLC method) under identical conditions. CAT attained the highest values in the diencephalon both in constant darkness and constant light. CAT dynamics counterbalances SOD evolution, in accordance with its dependence on SOD inhibitory effect (35). Equally in the diencephalon GPX reached peak levels in the dark and so did SOD in the light. As a rule, GPX activation in response to stress is accompanied by a drop in SOD and GSH-S-transferase activities. In parallel, GSH may also diminish (24). From the chronobiologic point of view the diencephalon is a most important nervous structure since it contains the suprachiasmatic nuclei (SCN), the biologic clock in mammals. Free radicals and related species such as NO and nitrate have already been supposed to generate or/and synchronize circadian rhythms in the SCN (31,3,30). Peroxides usually raise between 8 a.m. – 8 p.m. (max. 12 a.m.) and decrease at night (28,36), while GPX has its lowest values at 2 p.m. and its highest ones at 2 a.m. Melatonin CD release can participate in GSH, GPX and NO level control (16,17,20). After seven days spent in continuous light or continuous darkness, redox parameters tended towards normal values, except for MDA which raised again. This could stand for an adaptive response of the antioxidative systems and a persistent lipoperoxidation, suggesting

a biomolecular lesion. The adaptive behaviour had a circaseptan aspect and marked a heterostatic mechanism. Heterostasis corresponds to a stress challenged homeostasis. In this case it concerns the oscillatory radical level modulated by altered L:D cycles.

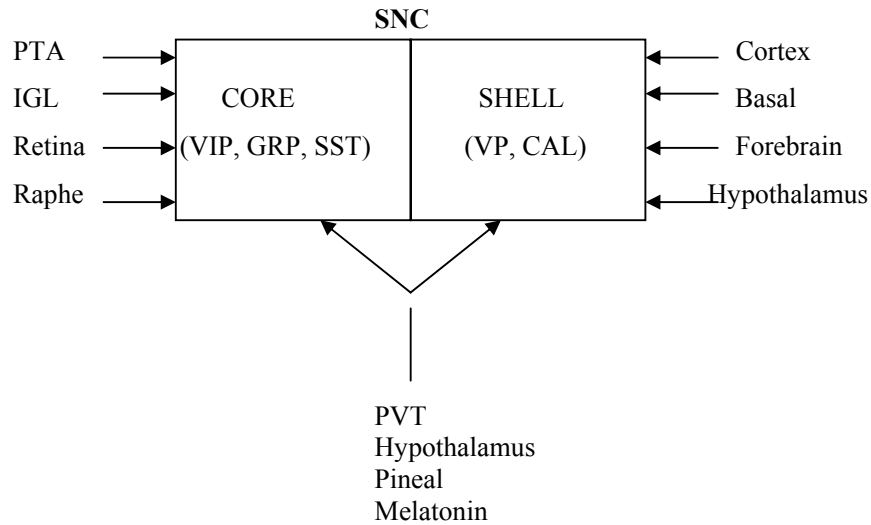
The functional phaseshifts of CD biorhythms along the photoneuro-endocrine axis (Fig.2), together with morphological cell injuries potentiate a biochemical deconditioning. Thus chronosusceptibility rhythms and anticipation response are impaired. Subsequently the illumination stress influenced the CD profile of the redox modulators and caused desynchronization of regulatory mechanisms.

The biochemical background of the oxidoreductive imbalance caused by light-dark cycle alteration could equally include the presence of possible **photosensitive neurotransmitters**. Rhodopsin e.g. has already been identified along the retino-hypothalamic tract in mammals (29). On the other hand melatonin level, an endogenous antistressor, antioxidant and humoral synchronizer, could be influenced. In fact, in rats, 1 lx represents the inhibition threshold for neural triggering. Inhibition peaks at 100 lx and the lighting regime in this study was of  $180 \pm 20$  lx. Stress elicited sympathetic nervous system stimulation, too.

Therefore a disruption of the CD behavioural coordination occurred. A complementary possible explanation of this process bears on endogenous and exogenous electromagnetic field interactions able to interfere with

electrochemical mediation and reactions. Cellular and subcellular coherence of electromagnetic oscillations

would play a part in molecular coupling or synchronization (7,17).



**Fig. 2. Overview of inputs to the suprachiasmatic nucleus (SCN)**

The SCN comprises two components: a) **the core**, including a population of vasoactive intestinal polypeptide (VIP)-, gastrin releasing peptide (GRP)- and somatostatin (SST)-containing neurons; b) **the shell**, including vasopressin (VP)- and calretinin (CAL)- containing neurons. Inputs to the SCN regions are segregated: primary and secondary visual perceptions from the retina, intergeniculate leaflet (IGL) and pretectal area (PTA) and raphe serotonin neurons go to the core, whereas inputs from the cortex, basal forebrain, brain stem and some hypothalamic areas go to the shell. Inputs from the paraventricular thalamic nucleus (PVT), other hypothalamic areas and melatonin mediation are to both core and shell (adapted after M.M.Moga and R.J.Moore, 1998).

Against this background a moderate stress induced ischemia-reperfusion syndrome enhanced  $O_2$  production and NO release (12).

The chronic oxidative stress (7 days) amplified initial desynchronization through an oscillatory response before determining adaptation to experimental conditions. The spatio-temporal versatility of such redox systems and

their electron dynamics integrated to oscillatory reactions could express an underlying biochemical cell clock vectorial mechanism. Impacting cell electrochemistry, exogenous and endogenous EMF interaction could, in fact, interfere with oxidoreductive cycles and hence with the electron dynamics of a possible free radical-dependent timing gear.

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Our experiments were carried out over two years. Therefore additional seasonal variations must be taken into account in their turn. In 72 h continuous dark entrained rats MDA values in the cortex were higher in March than in July while in the brain stem and moderately in the diencephalon they were lower in Spring than in Summer. Under 72 h continuous light, MDA was lower in April than in May in all the brain segments examined. For the same interval under continuous dark, SOD was higher in all the brain regions studied, while in constant light it had a smaller activity in March in the cortex and the diencephalon but greater in the brain stem. In 72 h continuous light synchronized animals GSH levels were more elevated in March than in September in the cortex and brain stem but up to four times lower in the diencephalon. In 48 h continuous light exposed animals GSH reached increased concentrations in the cortex and brain stem in September and trough ones in the diencephalon. In the cortex there was an up to two fold rise and the fall in the diencephalon was more than two fold. However, evaluation of circannual biorhythms of the redox balance was beyond the scope of this research. Undoubtedly, such influences correlate with natural light seasonal changes in the intensity, wavelength and frequency of its EMF, too.

### CONCLUSIONS

- An interdependence exists between light – dark cycle and redox balance
- in the CNS whose various structures investigated (cortex, brain stem, diencephalon, cerebellum) were differentially implicated in this adaptive regulatory mechanism.
- Free radicals, indirectly estimated by MDA levels, induced either the rise or the depletion of bioantioxidants (SOD, CAT, GPX, GSH) depending on the cerebral structure examined, the estimation timepoints (24 h, 48 h, 72 h, 7 days), and type of nyctohemeral cycle alteration (L:L or D:D).
  - Lipoperoxidation, expressed by MDA values, was more important under D:D than under L:L. Accordingly, the same was true of the antioxidative systems assessed.
  - These facts are in agreement with the increased nocturnal activity of rats and their innate CD structure.
  - An adaptive behaviour developed displaying a circaseptan aspect.
  - Seasonal variations of the cerebral redox balance were noticed.
  - Further investigations are needed in order to better define the specificity of these results and the electronic gear of a possible redox cellular clock.

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