Recovery of dopaminergic amacrine cells after strobe light stimulation in developing rat retina

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Introduction

- Concerns have been raised about the impact of strobe light on human health and life.
- Sources of strobe light include amplitude-controlled flashing lights, light-emitting diodes, and computer monitors, which can lead to visual discomfort, headaches, and poor visual performance.
- Dopaminergic amacrine cells (DACs) serve as the sole source of retinal dopamine, and dopamine release from the retina is regulated by light exposure following a circadian rhythm.
- Strobe light affects the number of DACs in development retina and retinal dopamine levels.

Purpose

- The aim of this study is to determine whether changes of dopaminergic amacrine cells by strobe light are recoverable after stopping strobe light during retinal development.

Material and Methods

- Animal model: Sprague Dawley rats
  - Control group: continuous light, from eye-opening (postnatal 2 weeks)
  - Strobe group: reared under strobe light for 2 weeks
- After postnatal 4 weeks, continuous light was given to all animals for recovery.
- Retinas were taken at postnatal 4, 6, 8 and 10 weeks.
- Evaluation of changes in the retina
  - Immunohistochemistry
    - Tyrosine Hydroxylase (TH): rabbit polyclonal antibody directed against TH (Merck Millipore), dilution 1:1000
  - Western blotting
    - TH, dilution 1:4000
    - β-actin: mouse anti-β-actin (Sigma), dilution 1:2000
  - High-pressure liquid chromatography (HPLC)
    - Dopamine (Sigma-Aldrich), 10 ppm
    - 3,4-Dihydroxyphenylacetic acid (DOPAC) (Sigma-Aldrich), 10 ppm
  - Retinas were viewed with a light microscopy (BX50, Olympus) and confocal microscopy (LSM 700, Carl Zeiss)
- The number of tyrosine hydroxylase-immunoreactivity (TH-IR) cells were measured in the image of whole-mount confocal microscopy.
- TH-IR cells with a soma area of less than 50 were measured as type II TH-IR cells, and soma area of 50 or more as type I TH-IR cells.
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Results

- **Morphology and number of type I and type II TH-IR cells in retina.**

Figure 1. Images of 1 µm-thick vertical semithin sections stained with toluidine blue in control (A) and strobe retina (B) at P10 weeks. There are no differences in thickness and other gross morphology. At P4 weeks, there is a difference in the number of type I TH-IR cells between the control and strobe retina. At P6 weeks, strobe retina type I TH-IR cells show a greater difference from control retina, but after 2 weeks the number of strobe retina type I TH-IR cells tends to increase again. Strobe retina type I TH-IR cells show recovery at P8 weeks (C). The number of type II TH-IR cells in the control retina tends to decrease overall but increases in P8 weeks. Even in the strobe retina, the number of type II TH-IR cells tends to decrease gradually, but the number of type II TH-IR cells rarely changes in P8 weeks. However, the differences between the populations are not significant (D). Difference were significant at *p < 0.05. Scale bar = 50 µm in A and B.

- **Distribution of type I and type II TH-IR cells by region.**

Figure 2. Type I and II TH-IR cells from P4 to 10 weeks showed distribution from nasal to temporal. Type I and II TH-IR cells are more distributed temporal than nasal in control and strobe retina. P6w control retina had significantly more type I TH-IR cells than the strobe retina. This difference is prominent in the nasal region, but there is no significant difference by region depending over time. Difference were significant at *p < 0.05.
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Figure 3.

The soma area of TH-IR cells was measured in the peripheral of the superior-temporal (ST) region of P6, 8, and 10 weeks. TH-IR cells were measured regardless of area and soma area was divided into 50 intervals. At P6 weeks, the number of TH-IR cells measured as the soma area of type I cells of the strobe retina is less than the number of type II cells. At P8 and 10 weeks, the number of TH-IR cells in the strobe retina is different from that of P6 weeks. In the strobe retina, the number of type II decreases, and the number of TH-IR cells measured by type I cells increases (A). Images from TH-IR cells in the periphery ST region of the postnatal 10 weeks control and strobe retina (B – G). In control retina (B, C, and D), only typical type I (arrow) and II (arrowhead) TH-IR cells are visible, but in the strobe retina (E, F, and G) of the P10 week, intermediate-sized (open-arrowhead) TH-IR cells are observed. Difference were significant at *p < 0.05. Scale bar = 50 µm in B and E. 20 µm in C, D, F, and G.

Figure 4.

Dopamine and DOPAC levels at the control and strobe retina P6, 8, and 10 weeks were analyzed using HPLC. At P8 weeks, the dopamine levels in the strobe retina decreased compared to that of the control retina (A). However, there was no significant difference at the DOPAC level (B). The density was measured after western blotting was performed with TH antibody on control and strobe retina at P6, 8, and 10 weeks. There was no significant difference in TH protein levels (C, D). The difference was significant at *p <0.05.

Conclusion

- Differences of number of DACs at P6w recovered after P8w by discontinuation of stimuli.
- Number of type II cells decreased while small type I cell or intermediate-sized cell increased at recovery period.
- These results suggests that changes of DACs by visual environment are reversible and can recover after discontinuation of stimuli.