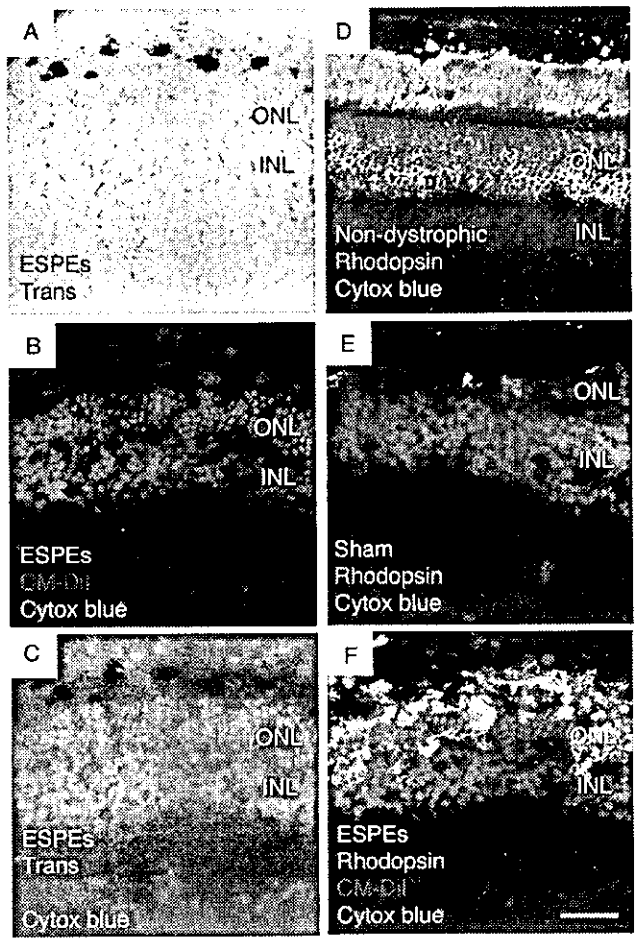

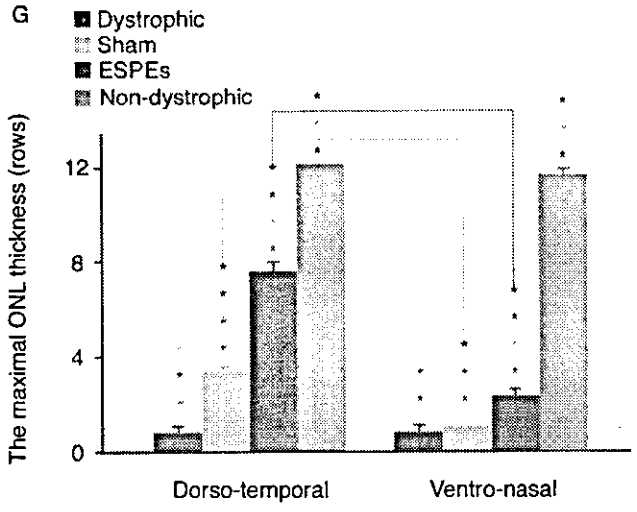

FIGURE 3. Immunohistochemical analyses after ESPE transplantation into RCS rats. (**A–C**) ESPE-grafted RCS rat retina at the age of 12 weeks (8 weeks after transplantation). (**D–F**) The expression of rhodopsin (*green*) in the photoreceptors at the age of 12 weeks. Congenic nondystrophic rat retina (**D**). Sham-surgery RCS rat retina (**E**). ESPE-grafted RCS rat retina (**F**). Nuclei in cells stained with Cytox blue (**B–F**, *blue*). The grafted ESPEs prelabeled with CM-DiI (**B**, **C**, **F**, *red*). (**G**) Histologic analysis at the age of 12 weeks. The maximum ONL thickness of the dorsotemporal retina (grafted quadrant) and that of the ventronasal retina (four animals in each group). Error bars, SD. *Colored asterisks*: statistical difference ($P < 0.05$, Mann-Whitney analysis) in the maximum ONL thickness compared with the nonsurgical RCS rat group (*red*), the sham-surgery RCS rat group (*blue*), the ESPE-grafted RCS rat group (*brown*), and the nondystrophic rat group (*green*). *Black asterisks*: significant difference ($P < 0.05$, Mann-Whitney analysis) in the maximum ONL thickness between the dorsotemporal and ventronasal retina. Scale bar, 80 μm.

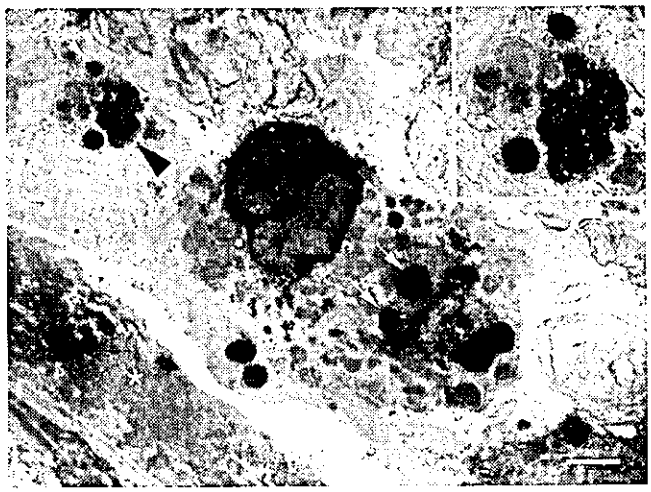

FIGURE 4. Electron microscopy of the grafted ESPEs. The presence of lamellar structures within the grafted ESPEs (*arrowhead*). *Inset*: higher magnification. *Arrows*: pigment granules in the ESPE cytoplasm. *Asterisk*: host RPE. Scale bar, 2 μm.

capability to promote the survival of photoreceptor cells in an animal model of RPE dysfunction.

Behavioral Assessment of Transplant Recipients

To determine whether the transplantation preserved visual function, each of the transplant-recipient animals was placed in a clear glass container surrounded by a motor-driven rotating drum with vertical black-and-white stripes (Fig. 5A). The visual function of the animals was estimated by measuring the head-tracking time in response to the rotating stripes, which is closely associated with the optokinetic reflex.[13] At the age of 12 weeks (8 weeks after the operation), the head-tracking time of the ESPE-grafted RCS rat group was significantly longer than that of the untreated RCS rat group (Fig. 5B; Mann-Whitney analysis, $P < 0.05$), but was not significantly different from that of the nondystrophic rat group. In contrast, the head-tracking time of the sham-treated RCS rat group was significantly shorter than that of the nondystrophic rat group (Fig. 5B, Mann-Whitney analysis, $P < 0.05$), but not significantly different from that of the untreated RCS rat group. These results indicate that ESPEs could preserve a significant level of visual function when transplanted into an animal model of RPE dysfunction.

Discussion

One of the advantages of using human ES cells for the treatment of degenerative diseases is that these cells have the capacity to provide an unlimited source of specific cell types. However, methods for purifying large numbers of lineage-specific cells should be developed for clinical application. In this study, we demonstrated that pigment epithelial cells can be generated, enriched, and expanded from primate ES cells. These ES-converted pigment epithelial cells showed development of several of the characteristics of RPE cells and were able

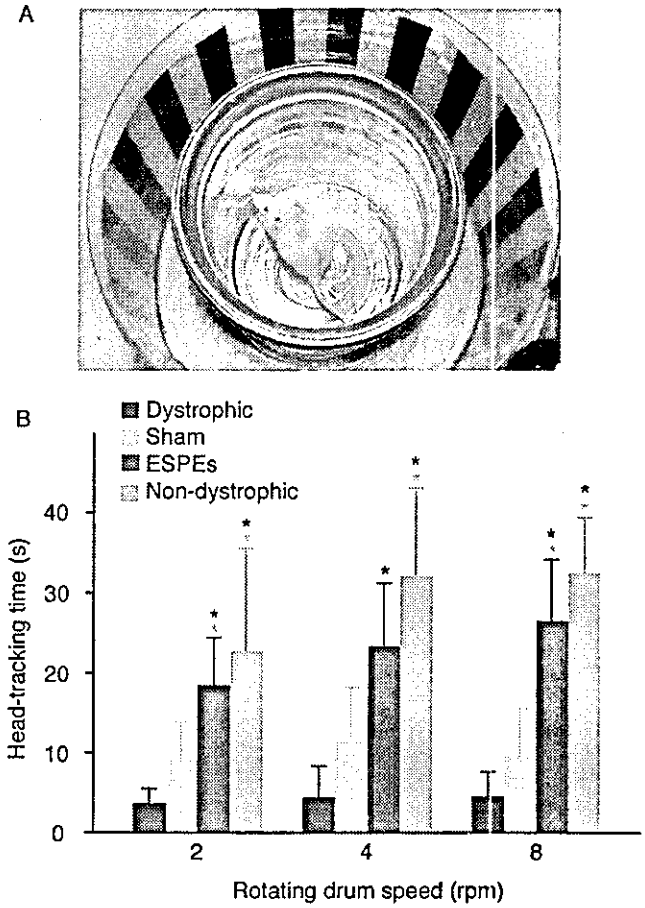

FIGURE 5. Behavioral assessment after ESPE transplantation into RCS rats. (A) Photograph of head-tracking apparatus. (B) The total amount of head-tracking time to rotating stripes at the speeds of 2, 4, and 8 rpm during the 4-minute test period for each speed (four animals in each group). Error bars, SD. *Asterisks*: significant difference ($P < 0.05$, Mann-Whitney analysis) in the head-tracking time compared with the nonsurgical RCS rat group (*red*) and the sham-surgery RCS rat group (*blue*).

to attenuate the loss of photoreceptors when transplanted subretinally into RCS rats.

The grafted ESPEs probably preserved the photoreceptors in the RCS rat retina, either by phagocytosing the host's outer segments[1] or by secreting soluble growth factors.[32] Although ESPEs are able to phagocytose latex beads (Fig. 2B), we have not measured their ability to phagocytose photoreceptor outer segments in vitro. However, phagosome-like bodies were seen in the grafted ESPEs by electron microscopy (Fig. 4), suggesting that grafted ESPEs had the ability to ingest host shed outer segments. The results of transplantation may have been even better if we could have transplanted an organized patch of ESPEs instead of dissociated cells, because, in such a patch, cellular polarity and tight junctions seem more likely to develop.

An earlier study reported the retinal transplantation of neural precursors that had been differentiated from mouse ES cells.[33] The transplanted cells probably slowed the photoreceptor degeneration in RCS rats by secreting some growth factors, because grafted neural precursors can neither phagocytose host shed outer segments nor differentiate into photoreceptor cells. In contrast, our results showed that ESPEs from primate ES cells can differentiate and develop characteristic properties of RPE cells, which would be necessary for the treatment of primary RPE dysfunction and for the long-term preservation of visual function after retinal transplantation.

If undifferentiated cells are contaminated, transplantation of ES cell-derived cells might involve the risk of tumor formation in the host animal. However, no tumors were observed in the animals that received ESPE grafts in our study. One reason for this may be that we selectively expanded the ESPEs as patches of cells on the matrix-coated dishes and generated relatively pure populations of donor pigment epithelial cells. These procedures may have kept unwanted cell populations from contaminating the donor cells.

Because both undifferentiated primate ES cells and ESPEs can be expanded in vitro, it is possible to generate an unlimited number of ESPEs for retinal transplantation. Considering the close phylogenetic relationship between humans and cynomolgus monkeys, we can also expect that the methods of differentiation used to generate ESPEs can be applied to human ES cells. Human retinal diseases for which ESPE transplantation may be used include age-related macular degeneration and hereditary retinal degeneration due to primary RPE dysfunction, such as some forms of retinitis pigmentosa.

One substantial problem to be solved is the control of immunologic rejection after the transplantation of allograft tissue. It is therefore important to determine in future studies how much immunosuppression is necessary after ESPE transplantation into the subretinal space, which is sometimes regarded as an immunologically privileged site for retinal allografts.[34] The transplantation of monkey ESPEs into the monkey subretinal space would provide a more accurate model for the allograft transplantation of human ESPEs into other humans.

Our results indicate that the expected morphologic, biochemical, and functional characteristics of RPE cells developed in the expanded ESPEs in vitro. After transplantation of the ESPEs into the subretinal space of an animal model of RPE dysfunction, the grafted ESPEs enhanced the survival of host photoreceptors. These effects were demonstrated both by histologic analyses and behavioral tests. To the best of our knowledge, this is the first study to show detailed functioning of specific cells differentiated from primate ES cells both in vitro and in vivo. In addition, this is also the first study to demonstrate the successful therapeutic application of primate ES cells in an animal disease model. As human ES cells significantly differ from mouse ES cells but closely resemble nonhuman primate ES cells, the latter would be more suitable for preclinical research aimed at cell-replacement therapies. Before human ESPEs are used in clinical trials, however, long-term studies of retinal transplantation in nonhuman primate hosts are necessary to confirm the cells' safety and efficacy.

Acknowledgments

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46. 視神経乳頭刺激型極細電極からの電流刺激による人工視覚の検討

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瓶井資弘、大路正人、八木哲也、田野保雄
(大阪大)

研究要旨　目的：人工視覚における視覚路電流刺激部位には大脳皮質、網膜上、網膜下などさまざまな部位が考えられているが、昨年の班会議での報告のように、我々は視神経乳頭もその1つになりうると考える。前回は、家兎を用いた急性実験によりその可能性を示した。今回我々は、より細い電極を用いてその長期的な有効性につき検討したので報告する。対象と方法：顕微鏡下にて極細白金電極（50 μm）を経硝子体的に家兎眼の視神経乳頭に刺入設置し、4〜6か月の経過観察期間中、網膜電図、視覚誘発電位、電気刺激による誘発電位を記録した。また経過終了後、電極周囲の視神経乳頭の変化を組織学的に検討した。結果：50 μm 径の白金線を視神経乳頭に安全に刺入設置することが可能であった。経過観察期間中、設置した電極総計20本のうち19本が視神経乳頭内に保持され、電流刺激による誘発電位の記録が可能であった。術直後の電流閾値は19.3 ± 9.2 μA (6.0 ± 2.9 μC/cm^2)であり、術後1か月では78.8 ± 31.9 μA (24.6 ± 10.0 μC/cm^2)と上昇したが、その後6ヶ月までは術後1ヶ月の値と有意な相違を認めなかった。網膜電図のa波b波および視覚誘発電位の潜時と振幅には、有意な変化を認めなかった。組織学的に設置した電極周囲に薄い線維膜を認めたが、電極の長期留置あるいは電流刺激によると思われる変化は認めなかった。**結論**：視神経乳頭設置型電極からの電流刺激の長期的な有効性、またその安全性が示されたことにより、当方式が、人工視覚システムの一方式となり得る可能性が示唆された。

A．研究目的

人工視覚における刺激方式には大脳皮質刺激、網膜上、下からの網膜刺激、我々が考案した強膜脈絡膜間、強膜内からの網膜刺激など、様々な方式が提案されている。我々は、その他に視神経刺激による人工視覚を考案している。前年度報告書では直径200μmの針型白金電極を用いた急性実験により当方式の有効性を示した。今回我々は、より細い電極を用いてその長期的な有効性につき検討したので報告する。

B．研究方法

刺激電極として直径50μmの極細白金電極を用いた。強膜創を作成し、それらから計4本の電極を挿入し、顕微鏡下にて硝子体鑷子を用いて先端部を視神経内に約0.5mm刺入した。術前、術後1ヶ月毎に、最長6ヶ月まで視神経電流刺激時における後頭部視覚野上の頭蓋骨に設置したネジ型埋め込み電極からの誘発電位EEPを記録した。電流刺激には複相型パルス刺激を用いた。
網膜、視神経の障害を調べるため、網膜電図ERG、視覚誘発電位VEPも術前、術後、測定した。また、経過観察後、眼球を摘出

し、組織学的に障害があるか否かを確認した。

C．研究結果

極細白金電極を安全に視神経乳頭内に設置することができ、埋植された計 20 本の電極のうち経過期間中 19 本が固定されていた（図１a)。蛍光眼底造影においても異常を認めなかった（図１b)。

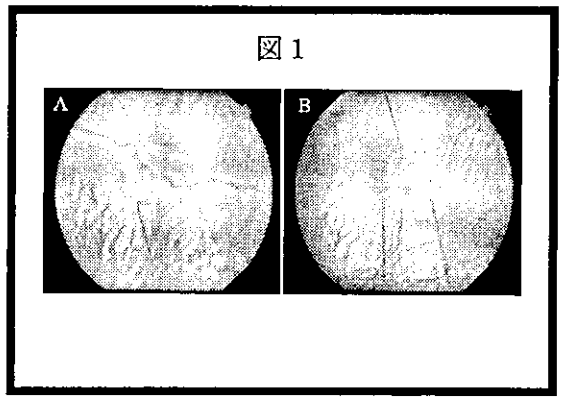

図１　埋植６ヶ月後の眼底写真と造影写真

術後経過観察期間を通じて ERG の a-wave, b-wave の電位、潜時には電極挿入眼と僚眼では差異を認めなかった（図２)。

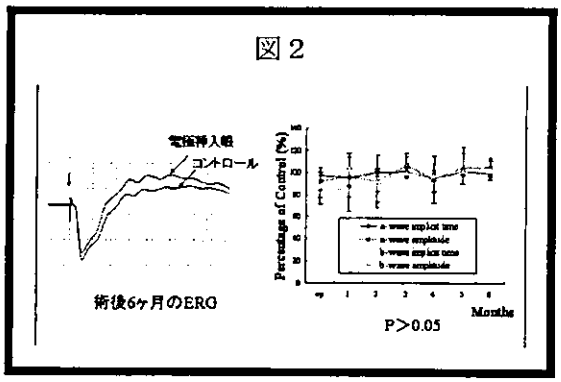

図２　経過観察期間における網膜電図 ERG

術後経過観察期間を通じて VEP の P－１波の電位、潜時には電極挿入眼と僚眼では差異を認めなかった（図３)。

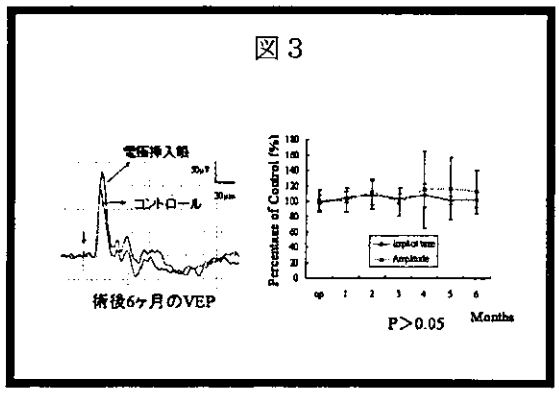

図３　経過観察期間における視覚誘発電位 VEP

誘発電位は経過期間中測定可能であり、誘発電位を生じさせる刺激電流の閾値は１ヵ月までは有意に上昇したが、以後は有意差がなくなった（図４)。

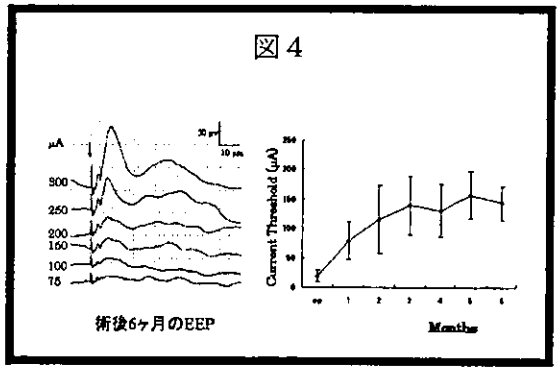

図４　経過観察期間における視神経刺激に伴う誘発電位 EEP　右は閾値を示す。

組織学的に、電気刺激によると思われる組織障害は認めなかったが、電極は結合組織で覆われていた（図５）。

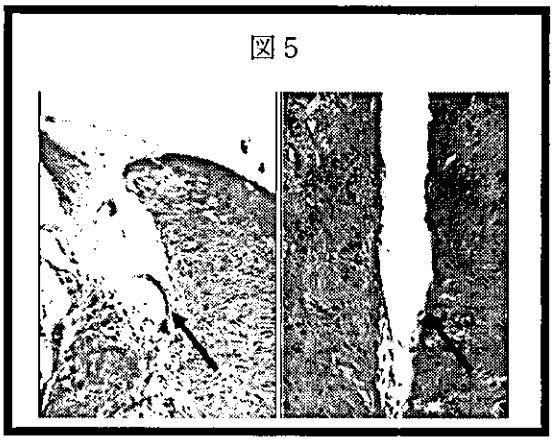

図５　電極付近における組織学的検索

D．考察

急性期のみならず長期の経過観察期間においても、視神経刺激型電極からの電流刺激により視覚野より誘発電位が得られたことにより、実際の患者においてもこの電極からの刺激により、誘発電位が長期に得られる可能性が高い。また２本の電極を用いた急性期実験と異なり、今回４本の電極でも誘発電位が得られたことにより、より分解能の高い視覚が得られる可能性がでてきた。

E．結論

視神経乳頭刺激型電極による視神経刺激は安全で有用な人工視覚の誘発法となり得る可能性が示唆された。

F．健康危険情報

なし

G．研究発表

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２．学会発表

第209回日本眼科学会総会にて発表予定。

H．知的財産権の出願・登録状況

１．特許取得

特許出願番号：特願2002-354330号
出　願　日　：平成14年12月5日
発明の名称　：「人工視覚システム」
発　明　者　：「田野保雄，不二門尚，
　　福田　淳，八木哲也」
　　以上４名
出　願　国　：日本

２．実用新案登録

なし

３．その他

なし

I．参考文献

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網膜脈絡膜・視神経萎縮症に関する研究
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