the location where the signal is produced should be carefully separated.

D Signals

The threshold of the D signal was comparable to that of the ERG b-wave in the dark-adapted condition (Fig. 2).[19] The threshold was higher by 2.0 to 3.0 log units under light-adapted conditions (Fig. 3),[17] and the amplitude was greatly decreased in the presence of background illumination (Fig. 5).[22,23] These results indicate that the D signal evoked by a dim flash (weaker than −5.0 or −6.0 log units) under dark-adapted conditions reflects the activation of rod photoreceptors, and those evoked by stronger flash reflects the activation of both cone and rod photoreceptors. The D signal evoked under light-adapted conditions reflects mainly the activation of the cone photoreceptors.

The time course of the D signal is slow and is probably produced by a flash-induced blood volume or flow increase. The decrease in light reflectance is due to the increased light scattering of the red blood cells. Previous studies have shown that flashing lights can increase the blood flow at the optic disc of humans and cats.[27,28] An increase in blood volume is known to decrease the reflectivity of tissues due to the increased scattering of light by the red blood cells in the blood vessels.[11,29] Although a change in the blood volume or flow was not directly measured in this study, the results of measurements of the intrinsic signals on the optic disc support this (Fig 6). In the central region of the optic disc occupied by the central retinal artery and vein, the reflectance changes were three times greater than in other regions, although the time course was almost the same at any locations within the optic disc. The light-scattering changes induced by changes in the blood volume or flow may be most effectively observed when the vessels are perpendicular to the imaging plane. Even in the white regions (Fig. 6A, Temporal and Nasal), where large vessels are not present, the light reflectance changes showed a time course similar to that in the central region (Fig. 6C) indicating that this signal is derived from the blood volume or flow changes in the capillaries. As for the question of whether the blood volume or flow contributes more significantly to this signal, we do not have any evidence to conclude which has the greater role and recommend that the mechanism of blood-related light scattering changes be thoroughly investigated.

Neural activity in the optic nerve causes shrinkage of the extracellular space due to cellular swelling, and this was detected optically by intrinsic signal imaging in rats.[30,31] We believe that part of the scattering changes may be due to swelling of the axons of the ganglion cells or of the glial cells. Its contribution to the whole intrinsic signal, however, may be masked by the relatively large reflectance changes due to changes in blood volume or flow. A contribution by the changes in deoxygenated hemoglobin concentration in the capillaries to the intrinsic signal may also exist, although it is believed to be negligible compared with that of tissue light scattering under infrared light observation.[9,11]

R2 Signals

The properties of the R2 signal were similar to those of the D signal, except that R2 became very small and in some cases became positive under light-adapted conditions (Figs. 3B, 5B). The R2 signal is probably a complex of different components and origins because the posterior retina is a complex layered structure, and its signal properties cannot be explained simply by the blood volume or flow changes in the capillaries.

We suggest that the inner retina may be the main contributor to the D and R2 signals because this type of slow signal was not observed at the fovea, which lacks the inner retinal layers including the blood vessels.[18] Our data did not allow us to determine which type of cells contribute the most to the flash-evoked responses observed at the optic disc or the posterior retina.

We attempted to keep the systemic condition of the anesthetized monkeys as constant as possible during the data acquisition. In some trials, however, the heart rate became unstable and rapid changes occurred during consecutive recordings under the same stimulus conditions. For example, the heart rate increased from 120 per minute to 140 per minute during two consecutive trials in one monkey. Although such data obtained under unstable conditions were discarded, we did note that it was always the amplitudes of D and R2 signals that were affected by the changes in the heart rate. In contrast, the amplitudes of F and R1 signals were much less affected by changes in heart rate (data not shown). This observation suggests that the D and R2 signals are related to blood-induced changes more than are the F and R1 signals.

It was interesting that the amplitudes of D and R2 signals were largest with 3- to 5-minute flash intervals (Fig. 4B). This finding is very different from the results of ERGs. It is possible that the mechanism by which neural activity is converted to the vascular response (i.e., neurovascular coupling)[32,33] is most effectively activated when the stimuli are given repeatedly at intermediate intervals. This possibility should be investigated more extensively.

F Signals

The F signal, which is the average of light reflectance changes within the central 300 μm in diameter was faster than the D and R2 signals and reaches its peak within 100 to 200 ms (Fig. 1C). The threshold for the F signal was much higher than any other signals and was the same in both dark- and light-adapted conditions. The characteristic anatomic structure of the fovea (viz., the absence of rod photoreceptors, capillaries and other inner retinal layers), indicates that the F signal reflects the activation of cone photoreceptors under any recording conditions.

The light-scattering changes due to the microstructural changes after activation of the cone photoreceptors are probably the source of the F signals because the foveal avascular region is free of capillaries and not subject to the changes in hemoglobin concentration or blood volume after neural activation.[18] Recent functional OCT studies using blood-free slice preparations showed that the reflectance in the photoreceptor layer is strongly changed by neural activation followed by microscopic morphologic changes.[34,35]

R1 Signals

The amplitude of the R1 signal increases with an increase in stimulus intensity under both dark- and light-adapted conditions as did the F signal. The threshold of R1 lies between the threshold for the optic disc and fovea and was the same under both dark- and light-adapted conditions. This leads us to think that cone photoreceptors mainly contribute to the R1 signal, because bleaching of rods in the bright condition did not change the R1 threshold. It is difficult to assume, however, that rod and cone photoreceptors play different roles in light reflectance changes.

The property of the R1 signal is complicated in another way. The abrupt darkening after a flash may well be explained by the photoreceptor responses like the F signal, but the results in Figure 4B strongly suggest that R1 share the same signal origin with D and R2 signals: The amplitude of R1 signal did not increase with longer interstimulus intervals, but attained a maximum with 3- to 5-minute intervals as with the D and R2 signals. We suggest that the R1 signal is produced not

TABLE 1. Properties of Four Components in Retinal Intrinsic Signals

	Signal Components			
	D	F	R1	R2
Time to the peak in amplitude	Slow	Fast	Fast	Slow
Threshold in dark adaptation (log unit) (ERG a-wave, −6.7; b-wave, −7.8)	−7.8	−1.8	−4.8	−7.8
Threshold shift in light adaptation	++	−	±	++
Increase in amplitude with shorter flash intervals (3–5 min)	+	−	+	+
Decrease in amplitude by light adaptation	+	±	+	++
Possible sources				
Contribution of blood-related light reflectance changes	++	−	?	++
Contribution of inner or middle layer	++	−	+?	+
Contribution of outer layer	−	++	+?	+?

only by photoreceptors but also by other inner or middle layer structures, although our data do not provide any evidence for the exact origin.

A summary of the various properties in four signal components is shown in Table 1.

Focal Stimulation

Focal stimulation of the retina is one way to evaluate local neural activity in a dysfunctional retina and has been applied clinically with the focal macular ERG.[24,25] The intrinsic signals measured with focal stimuli showed that this technique can also be used to study local responses. The focally stimulated region showed a decrease in the light reflectance after the stimulus, and this darkened region exactly matched the location of the focal stimulus (Figs. 7A, 7C). It was striking that the nonstimulated posterior pole showed a slow light reflectance increase after a fast light reflectance decrease (Fig. 7B; I). In another case, the nonstimulated posterior pole showed only a light reflectance increase (Fig. 7D; S).

The brightening observed in the nonstimulated region in later phase (Fig. 7B, I; Fig. 7D; S) may be explained by (1) some type of horizontal interaction by, for example, horizontal cells, through which stimulated neurons could affect the reflectivity of the neurons outside the stimulated region, or (2) the spatial interaction in the intrinsic signals between the stimulated and nonstimulated regions via an inhomogeneous distribution of capillary blood flow.[8,9,11,36] These explanations, however, do not account for the strong and homogeneous brightening over the whole posterior region triggered by a small focal stimulus.[37] It is possible that the properties of the signals, such as polarity and threshold, are different in different retinal layers, and the difference in signal time course between stimulated and nonstimulated regions would reflect the difference of layers that mainly contribute to the light reflectance changes.

Recently, OCT imaging of neural activity has been demonstrated in the feline visual cortex,[38] isolated frog and rabbit retina,[34,35] and intact rat retina.[39] Functional OCT studies in slice preparations have revealed the complex nature of flash-evoked changes in the reflectances from various intraretinal layers: a decrease of near infrared scattering in the photoreceptor layer and an increase in the ganglion cell layer,[34] or a decrease in the photoreceptor inner segment and increase in the internal plexiform layer and photoreceptor outer segments.[35] Srinivasan et al.[39] first reported the results of functional OCT signals in the intact retina and demonstrated the flash-evoked reflectance increase in the photoreceptor outer segments. In these studies, the increase in light reflectance after a flash was mainly observed in the photoreceptor layer, whereas the decrease was mainly observed in monkey and human retinas.[3,6] This difference in signal polarity may be attributable to the difference in the methods used to measure the reflectances. In addition, other factors, such as the use of sectioned preparations that lack the RPE layer and blood supply, differences among species, and differences in the recording region in the retina, should also be considered.

The light-scattering changes after a flash observed in functional OCT is thought to be derived from the structural changes in the outer segment discs, membrane hyperpolarization, cell swelling, and changes in the composition of the interphotoreceptor matrix.[34,35] These sources can also explain the rapid light reflectance changes (F and R1) observed in our study. As suggested by our results and those of functional OCT studies of retinal sections, the characteristics of the light reflectance changes after a flash are different in different layers and different retinal locations and may be far more complex than the conventional idea of intrinsic signals mainly investigated in the cerebral cortex.[8,9,11] Interpretation of the retinal intrinsic signal is thus difficult, and maximum care should be taken in choosing the proper recording conditions and which signal is most closely correlated with the neural activities of the retina.

In conclusion, our results showed that the intrinsic signals in the retina are composed of several components of different origins, although the precise cellular mechanisms of signal production were not determined. The sensitivity of intrinsic signal images was high enough to detect weak neural activity in the retina (e.g., the slow signals in the posterior retina and the optic disc were as sensitive as the ERG b-wave in the dark-adapted condition). Moreover, the distribution of intrinsic signals reflects not only the cellular distribution in the retina but the current level of the activities. Although the source of the signal was much more complex than initially thought, by carefully selecting the proper recording condition, this imaging technique may have a potential to estimate the neural responses of different origins and obtain more useful information about various types of retinal disorders with different etiologies than the conventional electrophysiological examinations such as full-field ERGs, focal macular ERGs, and multifocal ERGs.

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今月の話題

新しい網膜機能のイメージング法 —網膜内因性信号計測法—

風戸 陽子*1,2,3 谷藤 学*2 角田 和繁*2,3

光干渉断層計など眼科における画像診断技術は，近年めざましい進歩を遂げてきた。しかし，それらは解剖学的構造の把握を目的としており，視細胞をはじめとする網膜の神経活動を捉えることはできない。筆者らが開発した，新しい網膜機能のイメージング法である網膜内因性信号計測法について概説する。

はじめに

視機能の他覚的評価は，眼疾患の早期発見および治療効果の判定のために基本的かつ重要な課題である。眼科における画像診断技術（イメージング）は近年めざましい進歩を遂げてきた。例えば光干渉断層計(optical coherence tomograph：以下，OCT）は，検眼鏡によって捉えることのできない網膜微細構造の観察を可能にした。しかし，OCTや走査型レーザー検眼鏡(scanning laser ophthalmoscope：以下，SLO）などの画像診断法は，解剖学的構造の把握を目的としており，視細胞をはじめとする網膜の神経活動を捉えることはできない。そのため，網膜機能（神経活動）の他覚的評価のためには，電気生理学的検査である網膜電図(electroretinogram：以下，ERG)が今でも主要な役割を果たしている。

網膜，なかでもその中心に位置する黄斑部は，視力の維持のために重要な部位であり，同時に臨床的にさまざまな疾患が起こりやすい部位でもある。筆者らのグループでは，ERGとまったく異なる方法で神経活動を非侵襲的にイメージングする方法（網膜内因性信号計測法）を開発し，functional retinography（以下，FRG）と名付けた。そして，新たな眼科検査法としての実用化に向けた研究を行っている。

この技術により，従来は不可能であった錐体視細胞，桿体視細胞の機能的マッピングを高い空間解像度で行うことができるようになった。内因性信号計測法（intrinsic signal imaging）が眼底における機能計測に応用できれば，疾患の早期発見や早期治療につながることが期待される。

内因性信号計測法とは

神経活動に伴って神経組織の微細構造や光反射率が変化する現象は古くから知られている[1]。特に，神経活動に伴う代謝変化を光の反射率変化として捉える計測法は内因性信号計測法と呼ばれ，1990年以降この方法を用いて大脳皮質のさまざまな領域で機能的マッピングの研究が行われてきた[2~5]。

実際の計測法は，神経組織を charge coupled de-

New technique of functional retinal imaging—Functional retinography

＊1 *Yoko Kazato* 駿河台日本大学病院眼科 ＊2 *Manabu Tanifuji* 独立行政法人理化学研究所 ＊3 *Kazushige Tsunoda* 独立行政法人国立病院機構東京医療センター感覚器センター
別刷請求先：角田和繁（つのだ・かずしげ）〒152-8902 東京都目黒区東が丘2-5-1 独立行政法人国立病院機構東京医療センター感覚器センター

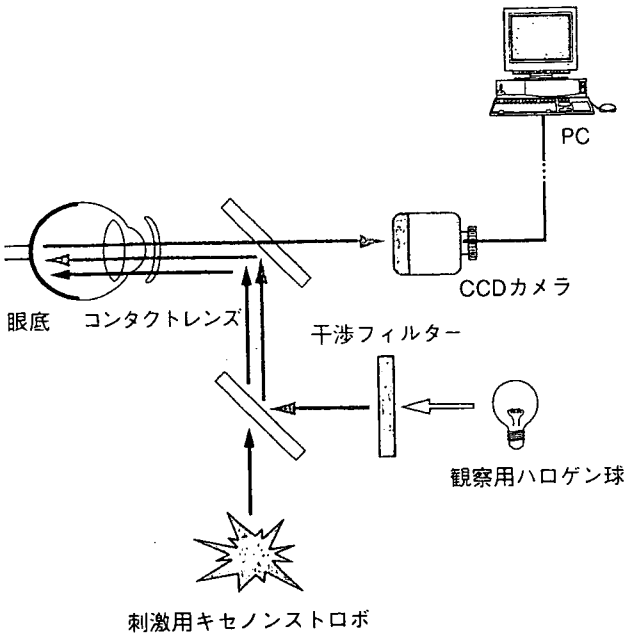

図 1　網膜内因性信号計測装置の概要

vice（以下，CCD）カメラで撮影し，刺激前と刺激後の画像を重ね合わせて比較するという非常に単純なものである。刺激後に画像の明るさが変化している部分が神経活動の起きた領域に相当し，通常は神経活動の高い領域が反射率の低下により暗く見える。信号の起源として主要なものには，神経活動に伴う組織の光散乱変化や血中ヘモグロビン飽和度の変化，毛細血管の血液量の変化がある。

ところで，大脳皮質において光学計測を行うためには脳表面を露出する必要があり，大きな侵襲を伴うものであるが，ヒトの網膜は内因性信号の計測に有利な特徴を備えている。まず，眼球の光学系（角膜，水晶体）を通して直接網膜を観察することができ，眼球全体が光学計測における理想的な観察用チェンバーとなるため手術的な侵襲がない。さらに，網膜外層では視細胞が密に一定方向に並んでいるため，光散乱変化による反射変化を捉えやすい。筆者らのグループではこの技術の眼科分野への応用に早くから着目し，網膜における実験を行ってきた[6~8]。

測定方法

FRG 開発のための動物実験では，ヒトの網膜とほぼ同じ解剖学的構造をもつニホンザル，アカゲザルを用いている。全身麻酔下において眼球運動を停止させ，眼底カメラを改良した観察系を用いて眼底を CCD カメラでモニターする（図 1）。

眼底観察用のハロゲン光は赤外線フィルター（840～900 nm）を透過して眼底後極部を照明する。眼底の観察に近赤外光を用いるのは，可視光で照明すると視細胞の色素褪色変化（bleaching）による眼底の光反射率の変化が起き，内因性信号と反対に明るくなる反応がみられるためである。解像度 640×480 pixels，毎秒 30 フレームの CCD カメラで眼底からの光反射率を持続的に記録する。測定開始から 0.5 秒後に眼底後極部全体を白色キセノンフラッシュ（1 ms）で刺激する。1 回の測定は通常 10 秒間行う。

刺激前 0.5 秒間の平均画像の反射率と，刺激後の画像における反射率との比をピクセル毎に計算し，その比を 256 階調にスケーリングし画像化する。

測定結果

1．明順応および暗順応下での網膜内因性信号

フラッシュによるびまん性刺激によって視細胞が活動すると，網膜全体の反射率が低下し画像では暗く描出される（図 2）。信号強度を疑似カラーで表示すると，明順応下では中心窩に内因性信号の急峻なピークを認め，周辺部に向かって減少するが（図 3a），暗順応下では中心窩に加えて周辺部にドーナツ状のピークを認める（図 3b）。内因

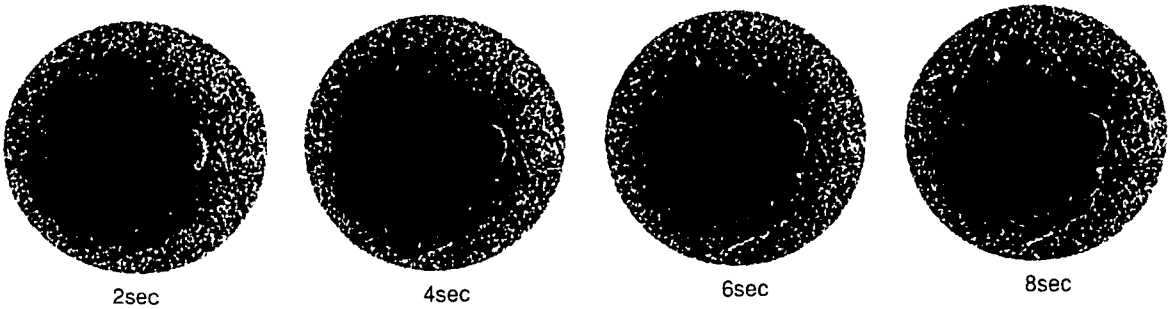

図 2　網膜内因性信号の時間経過

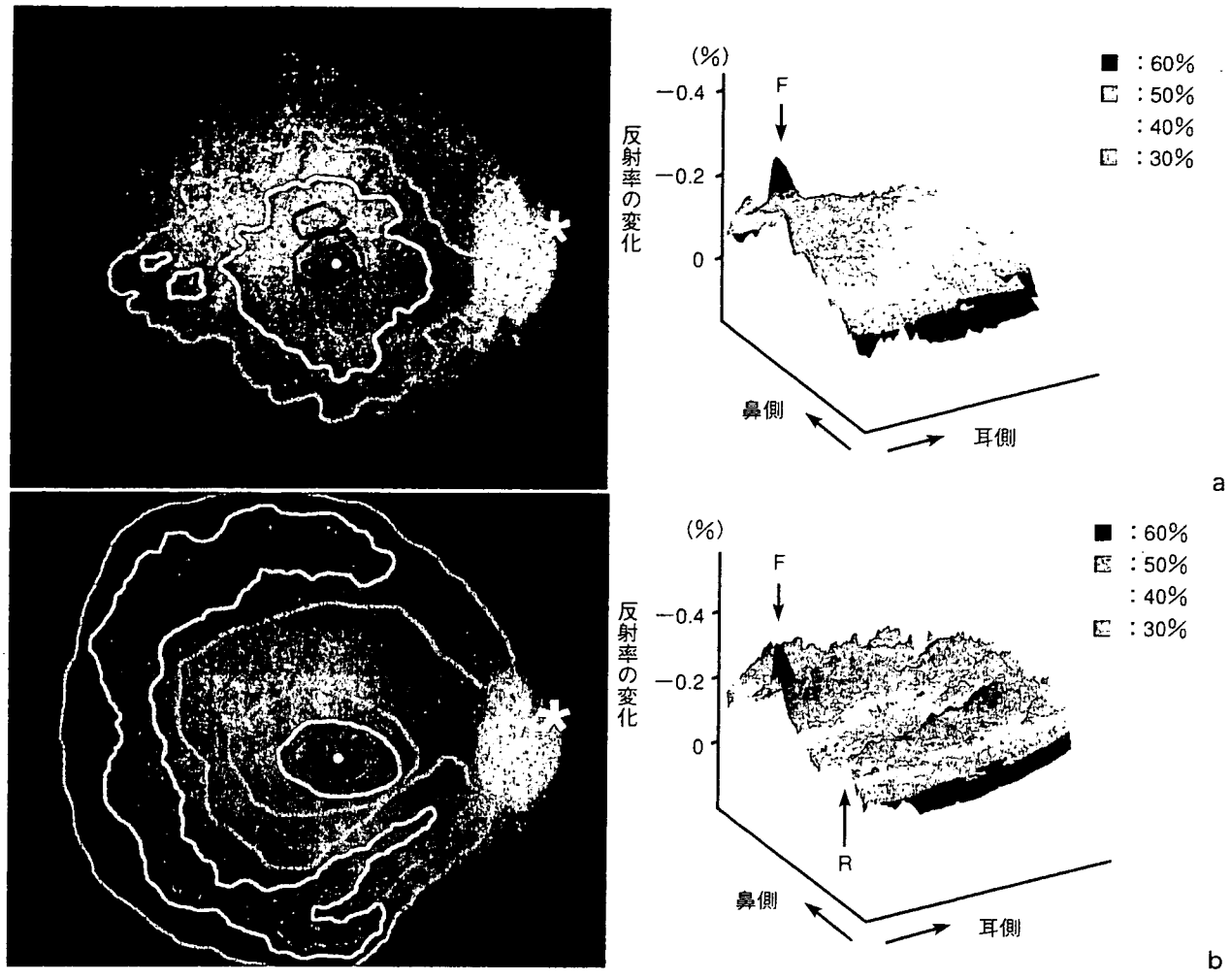

図 3　網膜内因性信号の 3 次元トポグラフィ
a：明順応下，b：暗順応下，＊：視神経乳頭部，F：中心窩，R：rod ring。

性信号のピークは中心窩では錐体視細胞に，周辺部では桿体視細胞の解剖学的な分布[9,10]（rod ring）によく一致しており，網膜内因性信号の発生には視細胞が大きく寄与していると思われる[6]。

2. 網膜部位と signal components

中心窩，後極部，視神経乳頭部の 3 か所で内因性信号を解析した（図 4）。図 5 にそれぞれの部位の反射率変化の時間経過を示す。

中心窩の反応（F signal，15×15 pixels）は網膜の中心，直径 300 μm の平均である。ここでは刺激後 100〜200 ms にピークをもつ早い反応がみられた。

次に視神経乳頭部に注目すると，ここでは網膜面とは異なりフラッシュ刺激後にゆっくりと信号が強くなり，5〜6 秒でピークに達する（D signal，70×50 pixels 以下）。これは刺激後の血流増加を反映した光散乱強度変化と考えられ[7,8,11]，中心動静

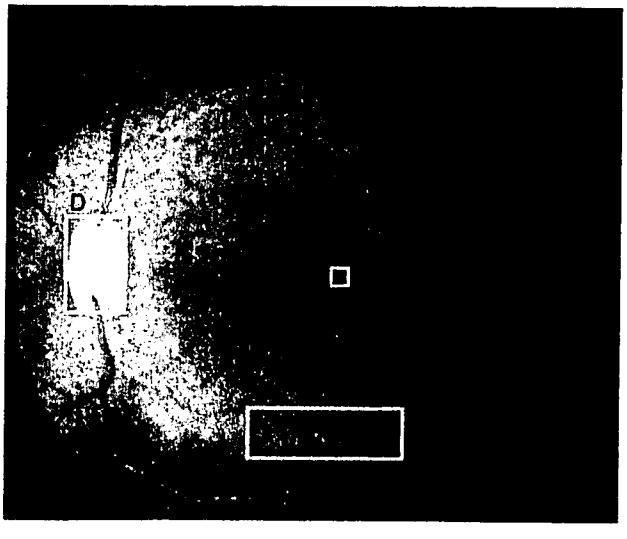

図 4　内因性信号解析部位
F：中心窩，R：後極部，D：視神経乳頭部。

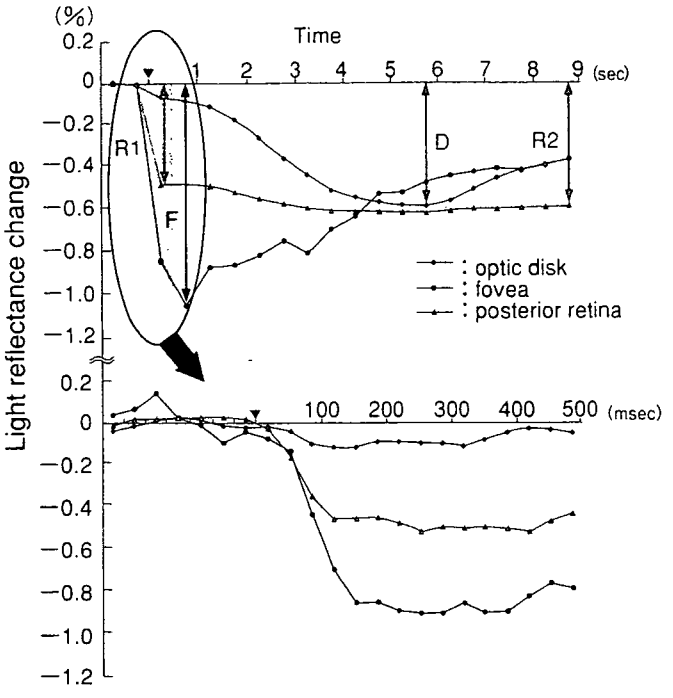

図 5　網膜内因性信号の時間経過

矢頭で示したフラッシュ刺激を 0 秒とした。下段にフラッシュ刺激から 500ms の時間経過を示す。F：中心窩の反応，D：視神経乳頭の反応，R1：後極部の早い反応，R2：後極部のゆっくりした反応を示す。

脈に相当する視神経乳頭中央部に急峻なピークがみられる（図 6）。

黄斑部を含まない後極部網膜の信号（60×40 pixels）は 100 ms 付近を屈曲点として，早い反応（R1 signal）とゆっくりした反応（R2 signal）に分けられた。これらの反応は中心窩を除く後極部全体で同じようにみられ，それぞれ主に網膜外層および内層の活動を反映していると考えられている[7]。

3. 網膜内因性信号と網膜電図の比較

角膜電極を用いて ERG を記録し，同一の刺激に対する FRG と比較した。

1）フラッシュ光強度と信号強度との関係（図 7）

暗順応および明順応の状態で，フラッシュ光強度を変化させていったときの網膜内因性信号，ERG の反応の大きさを比較した。

暗順応状態では，中心窩を除く網膜面の遅い反応と視神経乳頭部の反応閾値が最も低く，ERG の b 波の反応閾値とほぼ一致していた。それに対し，中心窩の反応域値は比較的高く，フラッシュ光強度の増加とともに反応も大きくなっている。臨床検査機器として確立している ERG と同程度に鋭敏であることがわかり，FRG のもつ可能性が改めて示された。

明順応状態では，網膜内因性信号，ERG とも暗順応状態と比べ，反応閾値が 2 log 程度高くなっている。しかし黄斑部の反応閾値のみ，暗順応状態と比較しても変化がない。これは，中心窩の内因性信号は網膜外層の錐体視細胞の神経活動を捉えているとする考えを支持している。

2）暗順応時間と信号強度との関係（図 8）

フラッシュ光刺激の間隔を変化させることで，暗順応時間と網膜内因性信号および ERG の反応の大きさを比較した。ERG では暗順応時間を長くすると a 波，b 波とも反応が大きくなり 30 分以降は一定となっている。網膜内因性信号では中心窩の反応は ERG と同じような時間経過をとるが，網膜面や視神経乳頭部の反応は 1〜3 分の暗順応時間で最も大きな反応を示す。その理由は明らかではないが，フラッシュ刺激により網膜の反応性の高くなる反応至適条件がある可能性がある。

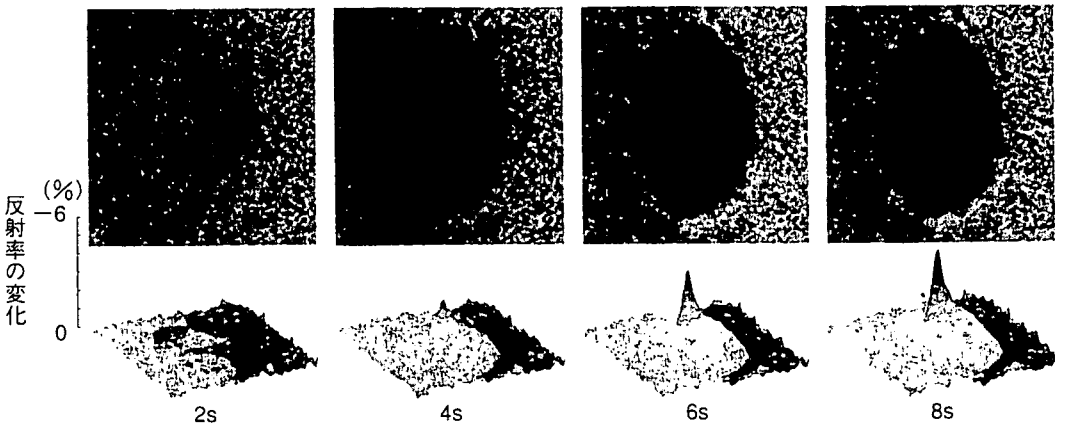

図 6　視神経乳頭部の内因性信号の 3 次元トポグラフィ

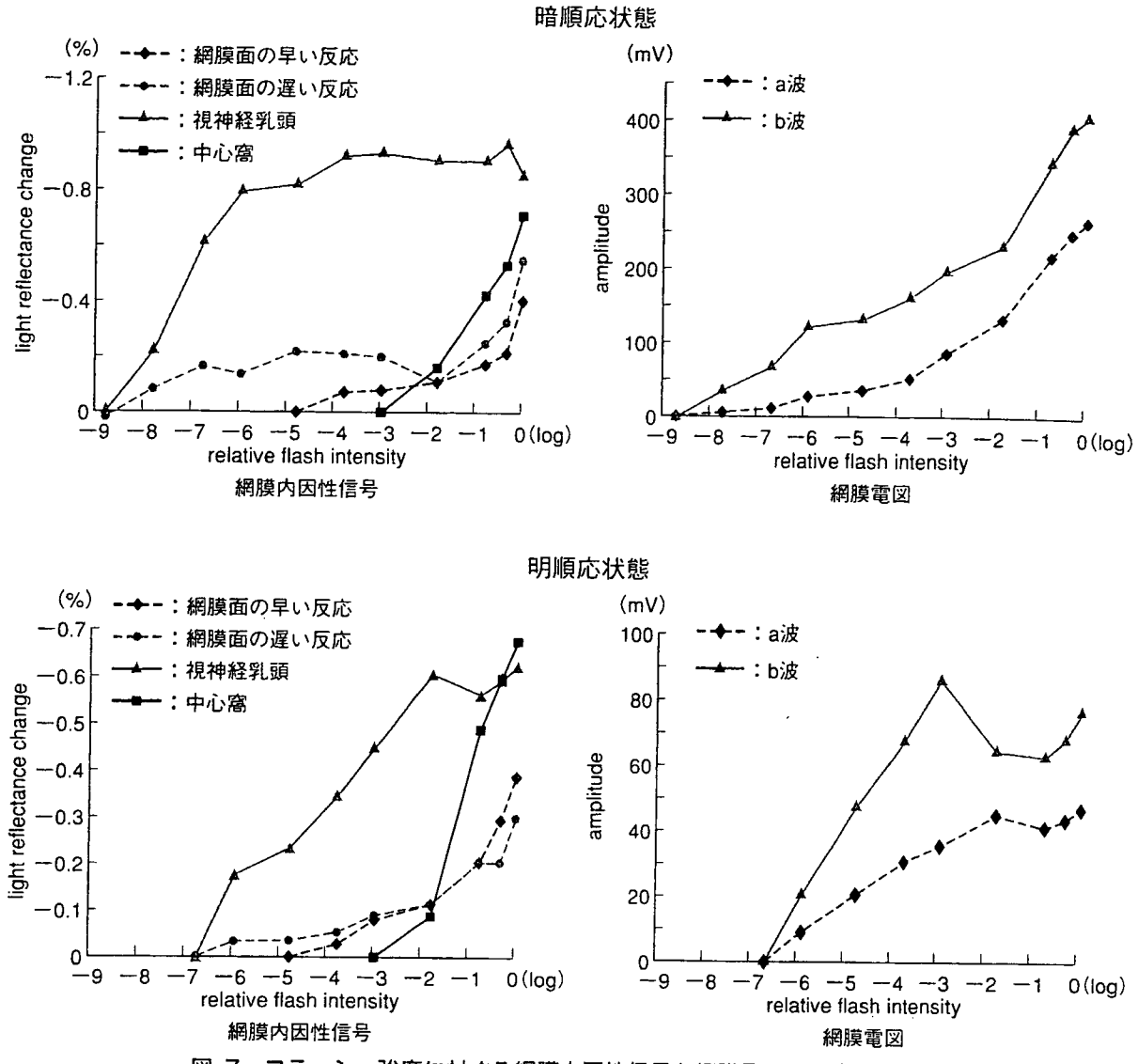

図 7　フラッシュ強度に対する網膜内因性信号と網膜電図の反応の大きさ

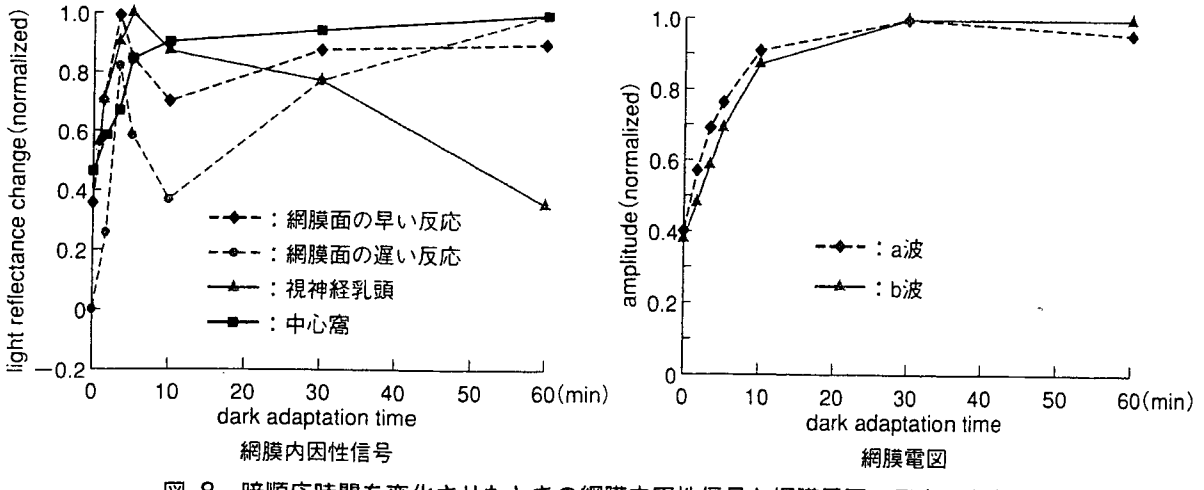

図 8　暗順応時間を変化させたときの網膜内因性信号と網膜電図の反応の大きさ

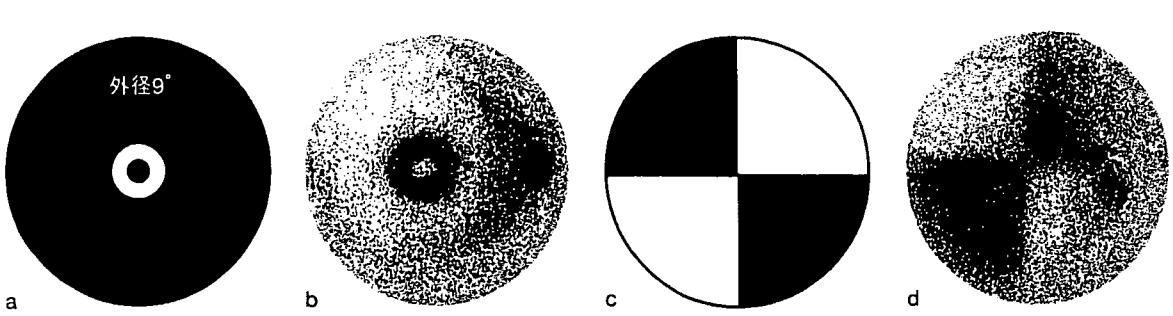

図 9　局所刺激による網膜内因性信号
a，c：フラッシュ刺激の形状，b，d：網膜内因性信号。

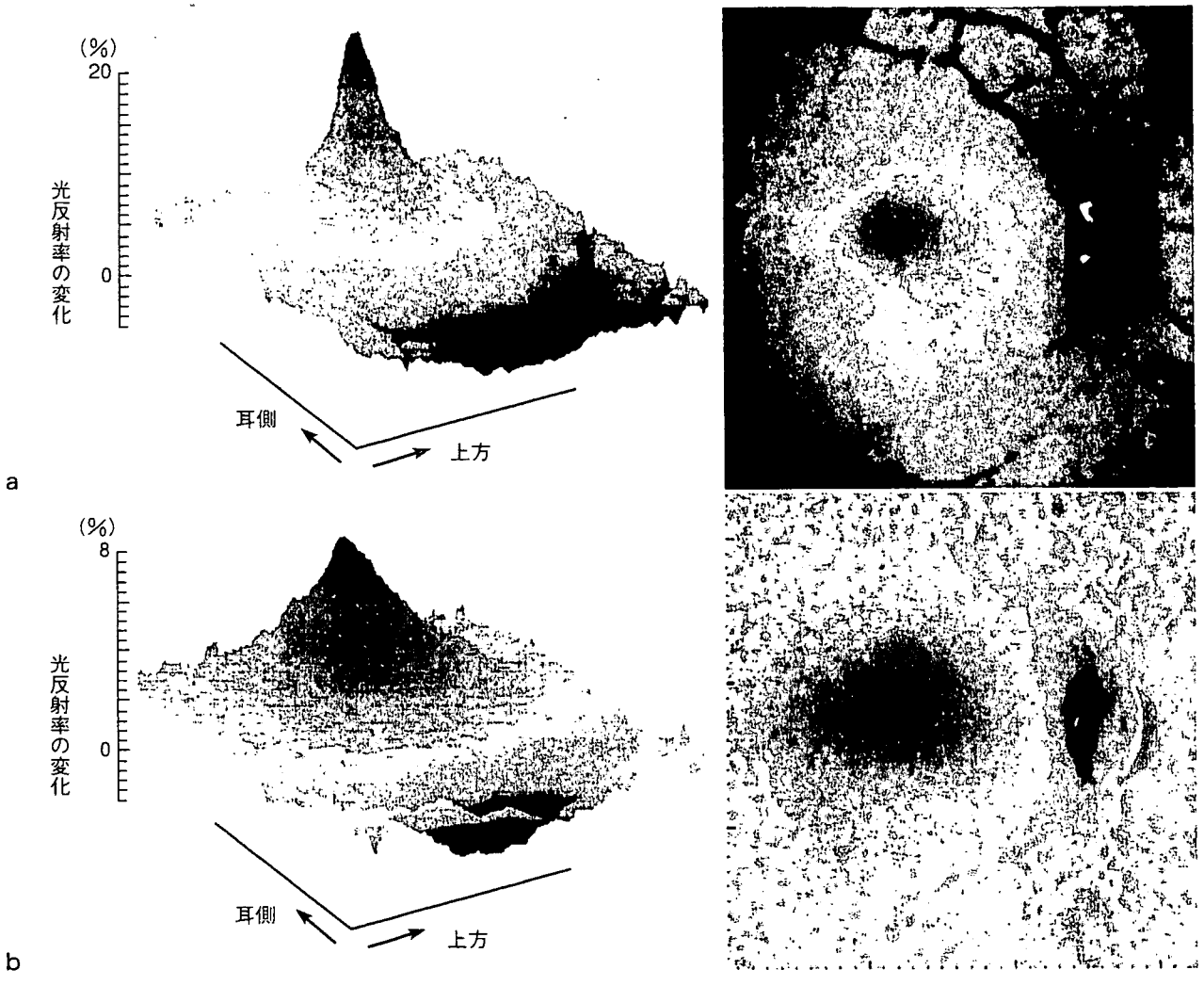

図 10　カラーフィルターを用いて測定した褪色変化のトポグラフィ
a：570 nm，b：630 nm。

さまざまな刺激方法による網膜内因性信号の測定

1．網膜局所刺激による網膜内因性信号（図 9）

網膜との共役面にフィルターを置いて局所フラッシュ刺激を行うと，刺激部位に相当する網膜から内因性信号を記録することができる。

2．可視光を用いた眼底撮影による網膜内因性信号

眼底観察光として可視光を用いることで視細胞外層の色素褪色変化（bleaching）を捉えることができる。これは，赤外光での内因性信号の測定とは異なり，褪色変化が起きた部分の網膜の反射率が上がる（明るくなる）反応であるが，570 nm の観察光では 20%（図 10a），630 nm の観察光では

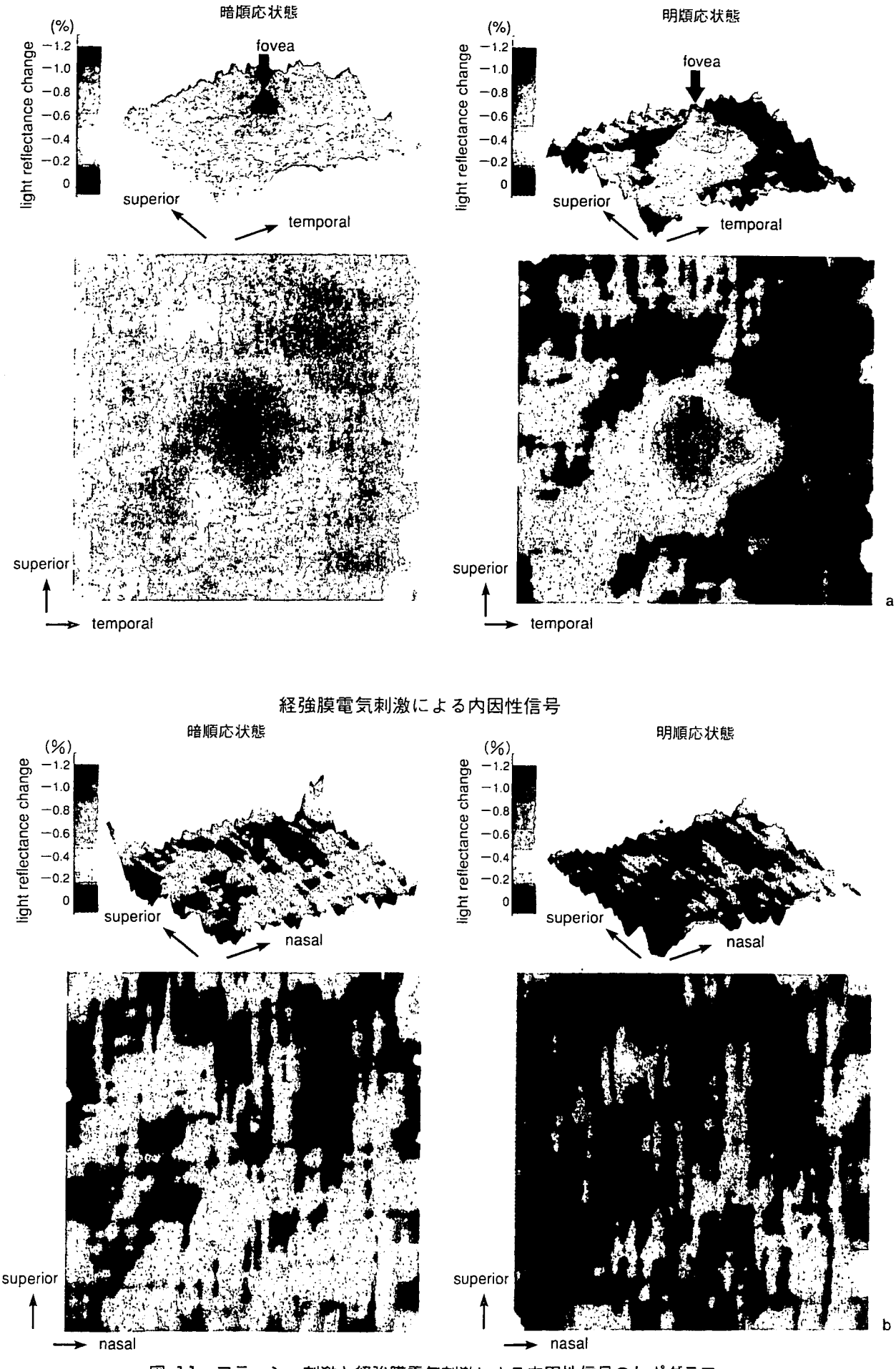

図 11　フラッシュ刺激と経強膜電気刺激による内因性信号のトポグラフィ

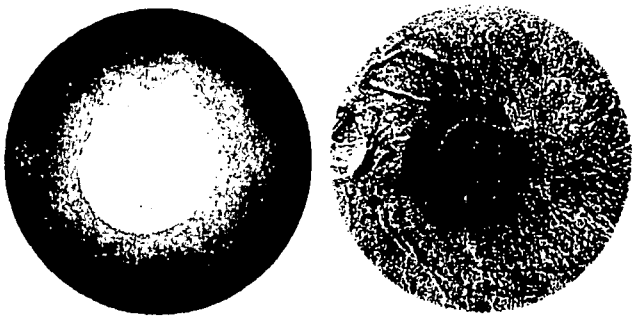

図 12　フラッシュ部分刺激に対する内因性信号
（ヒト網膜，覚醒下）

10%程度の大きな反射率変化が中心窩において認められる（図 10b）。

褪色変化自体は従来からよく知られているが，網膜内因性信号測定装置を用いることにより詳細なマッピングを得ることができる。この反応は信号の大きさが比較的大きく，信号起源が網膜視細胞であるとわかっているため，視細胞の機能評価として臨床応用に最も近いと考えられる。現在筆者らは，この信号の測定に特化した測定装置も作製しており，ヒトでの測定実験を行っている。

3. フラッシュ刺激と経強膜電気刺激による内因性信号（図 11）

では，フラッシュ光の代わりに電流によって網膜を刺激した場合はどのような反応が得られるのであろうか。経強膜的に網膜を電気刺激すると視神経および後頭葉視覚野の神経細胞が活動し，視野において白色の閃輝を自覚する。これは phosphene と呼ばれ，網膜外層の機能障害があっても内層の機能が保たれている場合には自覚することができる。このため phosphene の自覚（強さ，視野における範囲）を問診することによって網膜内層の障害をある程度推察することはできるが，実際に phosphene の程度を定量的に表現することは困難であり，生体網膜における刺激特性（反応閾値，刺激分布など）についての研究は少ない。

一方，経角膜あるいは経強膜的に網膜を電気刺激する治療法は，虚血性視神経症，網膜血管閉塞性疾患をはじめとする患者においてすでに国内でも試みられており，傷害された視野の改善をはじめとする治療効果が確認されている[12,13]。筆者ら[8]は経強膜電気刺激による網膜の反応分布を調べる目的で内因性信号を記録した。

二相性の経強膜電気刺激（current：500 μA, pulse frequency：20 Hz, stimulus duration：1.0 sec）により電気的に網膜を刺激すると，フラッシュ刺激でみられた中心窩の信号ピークがみられず（図 11a），網膜後極部全体でほぼ同程度の内因性信号がみられた（図 11b）。また，信号分布は明順応および暗順応の状態でも変わりはなく，信号発生の起源として網膜内層の寄与が大きいと考えられた[8,14]。

ヒト網膜における内因性信号測定

覚醒下のヒトでも，局所フラッシュ刺激により網膜内因性信号は測定可能であるが（図 12），現在のところ，詳細なマッピングを行うまでには至っていない。今後，測定技術および解析方法などのさらなる改良が必要である。

おわりに

FRG の利点は，赤外光の反射率変化を計測するために非侵襲的であること，空間分解能が高いこと，測定時間が短いことなどである。問題点は，ヒトの測定時に生じる固視微動などにより，画質が著しく低下することであり，現在それを克服すべく研究を行っている。将来，臨床応用が可能になれば，黄斑変性症や網膜色素変性症などさまざまな網膜疾患において精度の高い他覚的機能評価が可能になると期待されている。

さらに FRG 以外にも，近年は OCT を利用して神経機能評価を行おうという研究も行われている。これは 2002 年に理化学研究所の Maheswari ら[15,16]によって初めて提唱された Functional OCT という概念であり，網膜における応用に向けた研究が，Drexler，Fujimoto らなどすでに複数の研究者によって行われている[17,18]。網膜の神経機能をイメージングするという研究は新しい診断法として高く注目されており，将来は新たな網膜機能評価法として確立される日がくることが期待される。

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Distribution of Retinal Responses Evoked by Transscleral Electrical Stimulation Detected by Intrinsic Signal Imaging in Macaque Monkeys

Koichi Inomata,[1,2,3] *Kazushige Tsunoda,*[2,3] *Gen Hanazono,*[2,3] *Yoko Kazato,*[1,2,3] *Kei Shinoda,*[2,4] *Mitsuko Yuzawa,*[1] *Manabu Tanifuji,*[3] *and Yozo Miyake*[2,5]

Purpose. The distribution of the electrical current over the retina when electrical pulses are delivered transsclerally has not been clearly determined objectively and quantitatively in humans. The purpose of this study was to determine the pattern of electrically evoked neural activity in the monkey retina by using intrinsic signal imaging.

Methods. The intrinsic signals of monkey retinas were recorded as changes in the reflectance of infrared light from the retina after transscleral electrical stimulation by DTL electrodes. The effects of changing the stimulus parameters (e.g., intensity, duration, and frequency) of the electrical current, were investigated.

Results. Electrical stimulation evoked a uniform change in the reflectivity across the posterior pole of the retina; that is, the intrinsic signals changed uniformly. A peak of the intrinsic signal was not observed at the fovea. The threshold of the intrinsic signal was not significantly different for the macula, perimacula, and optic disc, and the threshold did not differ under dark- and light-adapted conditions. The strength of the signals increased with longer stimulus durations, and the maximum signals were obtained when the stimulus frequency was between 15 and 20 Hz.

Conclusions. Intrinsic signals of the monkey retina evoked by transscleral electrical stimulation are elicited uniformly across the posterior pole of the fundus and most likely arise from activation of the inner or middle layers of the retina. These functional measurements could serve as a diagnostic tool for mapping the inner retinal activity, by which the site of a lesion can be noninvasively imaged. (*Invest Ophthalmol Vis Sci.* 2008;49:000–000) DOI:10.1167/iovs.07-0727

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Electrical stimulation of the retina excites the neural pathway and evokes light sensations, called electrical phosphenes, and electrically evoked responses (EERs) from the occipital lobe.[1–4] Miyake et al.[5–9] have applied this technique for clinical diagnosis and have explored ways to evaluate the function of the inner retina of patients with total retinal detachment, night blindness, central artery occlusion, and optic nerve diseases objectively. With the increasing interest of retinal prosthesis,[10–12] the effects of electrical stimulation of the retina have drawn increasing attention because understanding the neuronal properties of the retina after electrical stimulation is essential for the development of retinal prosthesis[13,14] and determining residual retinal function in patients with inherited and acquired retinal degeneration should be known before a retinal prosthesis is implanted.[15,16] More recently, based on the results of several investigations on the neuroprotective effect of electrical stimulation,[12,17,18] electrical stimulation of the retina has been used to treat optic nerve diseases, such as nonarteritic ischemic optic neuropathy, traumatic optic neuropathy,[19] and longstanding retinal artery occlusion.[20]

Distribution of the electrical current across the retina, however, has not been definitively determined. Although electrical phosphenes are perceived homogeneously and continuously over the visual field when the retina is stimulated with a DTL electrode,[16] there is no way to evaluate the current distribution over the retina objectively, quantitatively, and noninvasively in humans. Electroretinographic techniques cannot be used to evaluate the retinal activity evoked by electrical currents because of the large electrical artifact from the stimulus.

Intrinsic signal imaging is a well-established imaging technique recently applied to translate neural activities elicited by photic or electrical pulses into the minimal visible changes of the appearance of the retina.[21–23] This has been used to assess the cone- and rod-induced responses in the retinas of macaque monkeys[24] and humans.[25,26] Recently, we reported that retinal intrinsic signals are composed of several components with different properties, and some of the components are strongly correlated with the neural activity of the inner retina.[27]

The purpose of this study was to determine the distribution of the electrical currents across the posterior retina after an electrical pulse. To accomplish this, we measured the changes in the retinal intrinsic signals evoked by transscleral electrical stimulation. A DTL electrode was used to deliver the electrical pulses, and different recording conditions, such as light- and dark-adapted states, along with different electrical current parameters, such as intensity, duration, and frequency, were studied. Results indicated that the electrical stimuli elicited responses uniformly across the posterior pole of the ocular fundus and activated mainly the inner or middle layers of the retina.

Methods

The principles of measuring intrinsic signals have been described in detail.[24,27] The experiments were performed on two rhesus monkeys (*Macaca mulatta*) under general anesthesia. After intramuscular injection of a mixture of atropine sulfate (0.08 mg/kg), droperidol (0.25 mg/kg), and ketamine (5 mg/kg), the monkeys were paralyzed with

From the [1]Division of Ophthalmology, Department of Visual Science, Nihon University School of Medicine, Tokyo, Japan; [2]Laboratory of Visual Physiology, National Institute of Sensory Organs, Tokyo, Japan; [3]Laboratory for Integrative Neural Systems, Brain Science Institute, RIKEN, Saitama, Japan; [4]Department of Ophthalmology, Oita University Faculty of Medicine, Oita, Japan; and [5]Aichi Shukutoku University, Aichi Japan.

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Corresponding author: Kazushige Tsunoda, Laboratory of Visual Physiology, National Institute of Sensory Organs, Tokyo. Japan, 2-5-1 Higashigaoka, Meguroku, Tokyo 1528902, Japan; tsunodakazushige@kankakuki.go.jp.

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vecuronium bromide (0.15 mg/kg per hour) and were artificially ventilated with a mixture of 70% N_2O, 30% O_2, and up to 1.5% isoflurane. Electrocardiograms, expired CO_2, and rectal temperatures were monitored throughout the experiments. Before the recordings, one pupil was fully dilated with topical tropicamide (0.5%) and phenylephrine hydrochloride (0.5%). A hard contact lens was placed on the eye to prevent the cornea from drying.

The experimental protocol was approved by the Experimental Animal Committee of the Riken Institute, and all experimental procedures conformed to the guidelines of the Riken Institute and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Intrinsic Signal Imaging

A digital fundus camera system (NM-1000; Nidek, Aichi, Japan) was modified to record the light reflectance changes of the macaque retina over 45° of the posterior pole. The fundus reflectance was continuously monitored with light from a halogen lamp bulb filtered through an infrared interference filter (840–900 nm). The camera was focused on the macular vessels, and the images were recorded with a charge-coupled device (CCD) camera (PX-30BC; Primetech Engineering, Tokyo, Japan). The images were digitized with an IBM-compatible computer equipped with a video frame-grabber board (Corona I; Matrox, Quebec, Canada: gray level resolution, 10 bits; spatial resolution, F1 640 × 480; temporal resolution, 1/30 second; Fig. 1A). The respirator was stopped during the recordings to reduce the respiration-induced motion artifacts.

For recordings in the light-adapted condition, half an 80-mm diameter white polyethylene ball was placed between the fundus camera and the eye. The ball was illuminated by two halogen lamps through AQ: 2 fiber optics so that the luminance in the center was 30 cd/m^2. The ball was removed a few seconds before data acquisition.

Electrical Stimulation of Retina

DTL electrodes were used to deliver the electrical pulses. The DTL electrode was selected for the measurements of intrinsic signals because the noise of the intrinsic signal is much lower with a DTL electrode than with a contact lens electrode such as the Burian-Allen electrode and because electrical phosphenes were perceived to be more homogeneous and continuous with a DTL electrode than with a contact lens electrode.[16]

The fibers of the DTL electrode were placed on the lower bulbar conjunctiva 5 mm from the corneal limbus. The conjunctiva was covered with 3% hyaluronic acid and 4% chondroitin sulfate (Viscoat; Alcon Japan, Tokyo, Japan), and the reference electrode was placed on the ipsilateral wrist (Fig. 1A).

Biphasic electrical pulses were used for all experiments (Fig. 1A, inset). The stimulus consisted of a positive current for x msec followed by a negative current for x msec and then a rest period for $3x$ msec. In most of the experiments with a pulse frequency of 20 Hz, x was set to 10, but in the experiment in which the pulse frequency was changed, x varied from 40 (5 Hz) to 2 (100 Hz) to keep the total current constant.

Stimuli were delivered 0.5 second after the initiation of data acquisition for 1 second, except in the experiment in which stimulus duration was changed from 0.5 second to 7 seconds. Pulse duration and frequency were controlled by a function generator (Multifunction Synthesizer WF 19443B; NF Corporation, Yokohama, Japan). Timing of the data acquisition and stimulus delivery were under computer control.

To compare the responses evoked by light flashes, white light stimuli were obtained from a xenon strobe (duration, 1 msec) embedded in the fundus camera. The light stimulus was given to the entire posterior pole of the ocular fundus, 0.5 second after the initiation of data acquisition. Flashes were delivered either as flickering flashes (20 Hz, 1 second, Fig. 2A) or as a single flash (Fig. 3). Flash intensity F2 F3 measured at the cornea was 6.07 cd · s/m^2 for the flickering flashes, and 140 cd · s/m^2 for a single flash (measured at 50.2 mm from the object lens by a photoradiometer; IL-1700, International Light Technologies Inc., Peabody, MA).

Data Analyses

After electrical or light stimulus, the light reflectance from the ocular fundus decreased and the fundus image became darker. The intrinsic

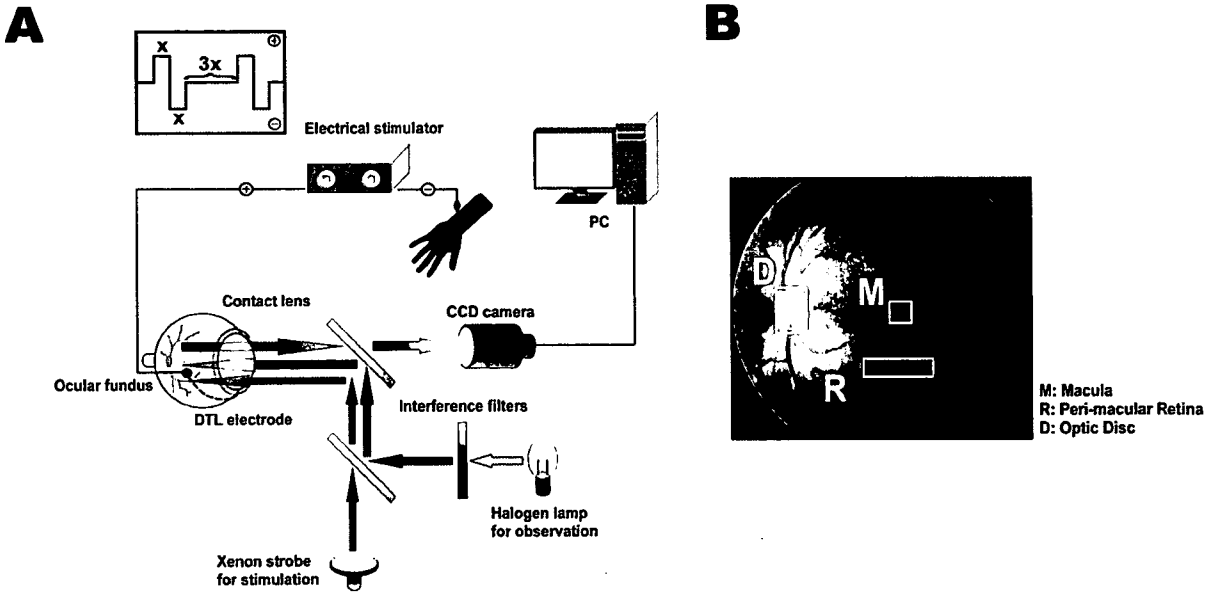

FIGURE 1. Schematic drawing of the experimental setup, pattern of electrical pulses, and fundus photograph. (A) Ocular fundus was illuminated by the light from a halogen lamp filtered through an infrared interference filter and monitored by a CCD camera during the recording trial. Stimulus was given by a xenon flash through the objective lens of a fundus camera or by electrical current through a DTL electrode placed on the conjunctiva. *Inset*: biphasic pulse current with a duration of x is followed by a resting period as long as $3x$. As the frequency is changed from 5 to 100 Hz, x is changed from 40 to 2 msec, respectively. (B) Fundus photograph of normal retina showing the locations to be analyzed.

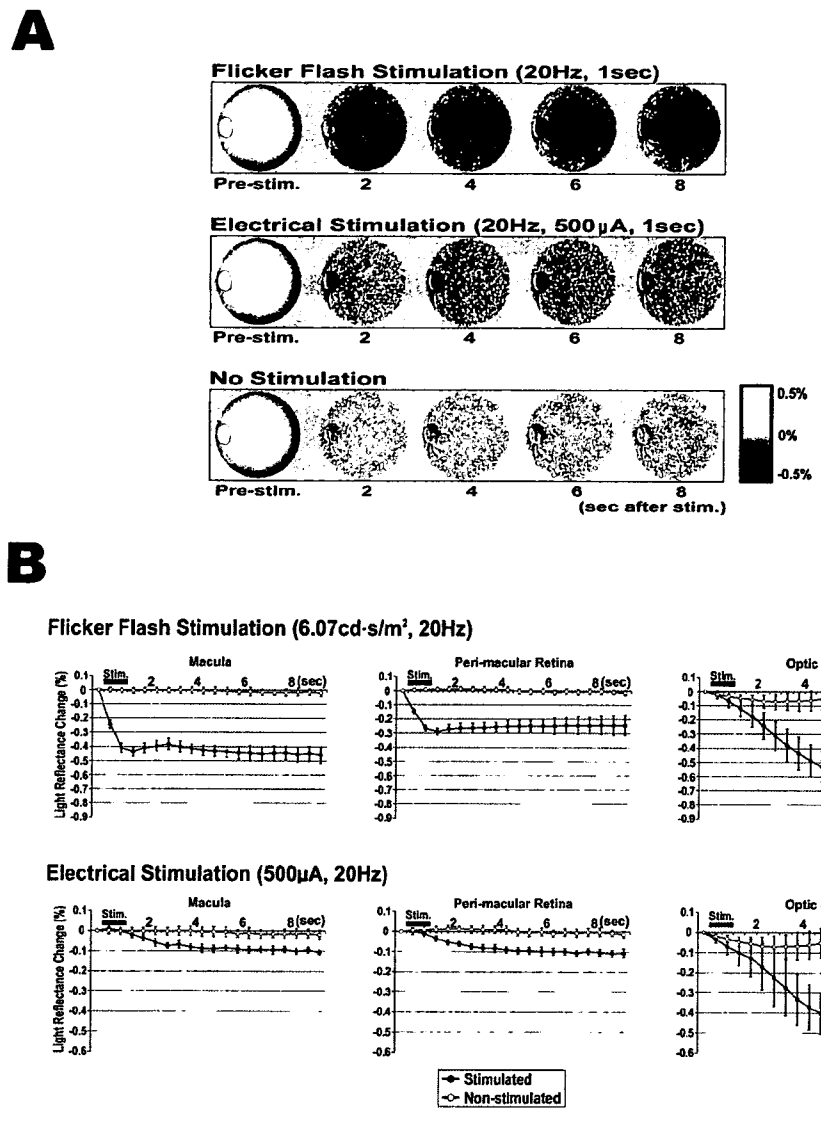

FIGURE 2. Time courses of flash-evoked and electrically evoked intrinsic signals. (A) Monochromatic infrared images of the ocular fundus showing the light reflectance changes during 10 seconds with flicker flash stimulation (*top*) or electrical stimulation (*middle*) or without stimulation (*bottom*). Images on the *left* are fundus images taken before the stimulation. Images on the *right* are the differential images showing the light reflectance changes after stimulation. Thirty consecutive video frames collected during 1 second were averaged for one poststimulus fundus image. Darkened regions indicate a decrease of light reflectance after the light stimulus. The data of three consecutive trials are averaged. (B) Plot of time courses of the light reflectance changes, evoked by flash (*top*) and electrical (*bottom*) stimulation at three different regions in a normal eye. The period of stimulus delivery (1 second) is indicated by *thick bars*. The time after the initiation of stimulus is shown on the abscissa. Data of 10 consecutive trials were averaged.

signals were measured as the stimulus-evoked changes in light reflectance. The amplitude was calculated as poststimulus grayscale values/prestimulus (0.5-second period) values pixel by pixel. This ratio was rescaled to 256 levels of grayscale resolution to show the stimulus-induced reflectance changes (Fig. 2A).

Each recording trial consisted of 300 video frames collected at 30 frames per second for a total recording time of 10 seconds The grayscale values of 15 video frames collected in 0.5 second were averaged for individual data points to determine the time course of the flash-induced reflectance changes (Fig. 2B).

In our previous studies, we showed that the response properties of the intrinsic signals evoked by a brief light flash were distinctive for different regions of the ocular fundus because they arise from different neuronal and vascular components of the eye, though the precise cellular mechanisms of signal production have not been determined.[24,27] To compare the electrically evoked signals with the light-evoked signals, three retinal regions were examined: the macula (30 × 30 pixels, covering 3.5° of the center), the perimacular region between the macula and the inferior-temporal artery (95 × 25 pixels), and the optic disc (40 × 60 pixels; Fig. 1B). To plot the time courses of reflectance changes, grayscale values within each region were averaged (Fig. 2B).

RESULTS

After flickering light or electrical stimuli, the light reflectance of the posterior retina and the optic disc decreased and the image of the ocular fundus became darker (Fig. 2A, top and middle). The time courses of the intrinsic signals, however, were different for these two stimuli. The time courses of the signals in three regions evoked by light flashes (20 Hz, 1 second, 6.07 cd · s/m^2) and electrical pulses (20 Hz, 1 second, 500 μA) under dark-adapted conditions are shown in Figure 2B. With flickering light, the reflectance changes in the macula and the perimacular retina were more rapid than at the optic disc, with the signal reaching its negative peak within 1.5 seconds after the flash. With electrical stimulation, on the other hand, light reflectance changes in the macula and the perimacular retina were as slow as those at the optic disc. Although the onset of light reflectance changes in the perimacular retina slightly preceded that in the optic disc, the signals in three regions reached their negative peaks 5 to 6 seconds after the stimulus. This trend in the signal time course was the same regardless of the current intensity, for a range of 100 to 1000 μA (data not shown).

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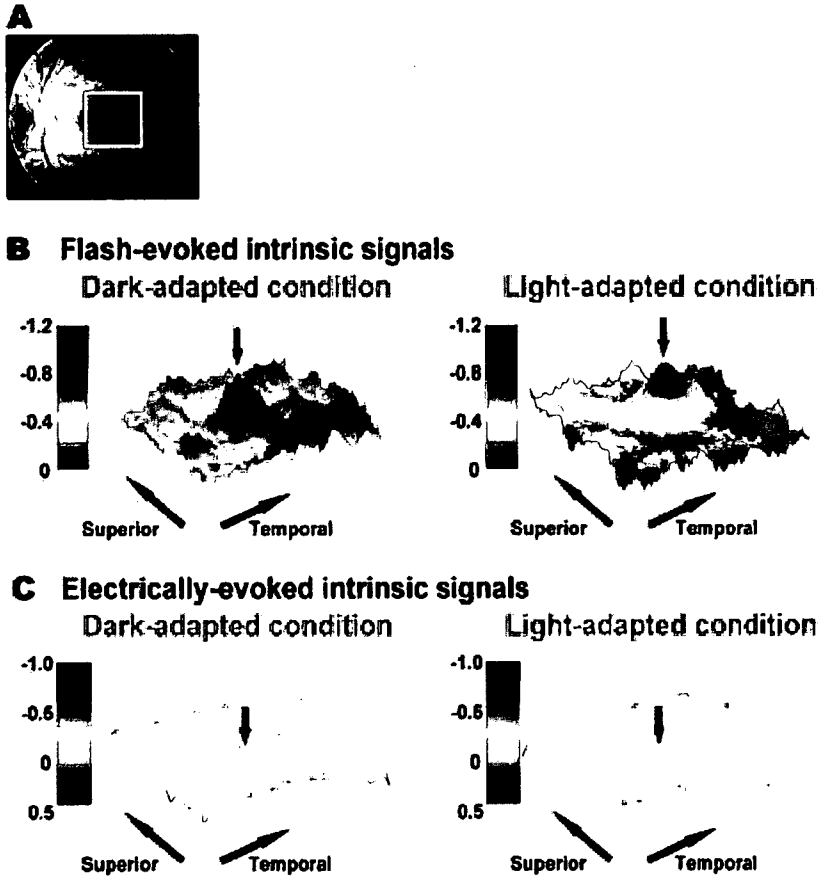

FIGURE 3. Topographic maps of the intrinsic signals elicited by flash-evoked and electrically evoked stimuli in the posterior retina. Pseudocolor topographic maps of light reflectance changes after a single flash (B; flash intensity, 140 cd · s/m^2; duration, 1 msec) or electrical stimulus (C; current, 500 μA; pulse frequency, 20 Hz; stimulus duration, 1 second) in the posterior pole of retina, under dark (*left*)- or light (*right*)-adapted conditions. Twenty consecutive trials were averaged to construct the topographic map. The location of the fovea is indicated by *red arrows*. The region of interest is shown by a *white rectangle* in (A). Note that negative values of light reflectance changes are plotted to indicate the strength of intrinsic signals at each locus.

Spatial Distribution of Intrinsic Signals

Distribution of the intrinsic signals evoked by a flashed light stimulus represents the responses of cone and rod photoreceptors.[24] After 30 minutes of dark adaptation, a topographic map of the intrinsic signal elicited by a flashed light stimulus had a steep peak at the fovea, and the perimacular region was moderately activated (Fig. 3B, left). The strong response at the fovea reflects cone-induced activities, and the response at the perimacular region reflects both cone- and rod-induced activities.[24] In the light-adapted condition, the topography of the response had a steep peak at the fovea, but the response in the perimacular region was strongly reduced because of suppression of rod function (Fig. 3B, right).

The distribution of the electrically evoked signals, on the other hand, did not have a foveal peak in dark- or light-adapted conditions (Fig. 3C). In addition, the perimacular response under dark-adapted conditions did not differ significantly from that under light-adapted conditions. The intrinsic signals evoked by electrical stimulation were roughly homogeneous in the posterior pole, and the spatial distribution did not reflect the anatomic distribution of cone and rod photoreceptors as it did with light stimulation.

Effect of Changes in Stimulus Current

The effect of currents ranging from 0 to 1000 μA on the intrinsic signals was determined under dark- and light-adapted conditions (pulse frequency, 20 Hz; stimulus duration, 1 second; pulse duration, 10 ms; Fig. 4). The peak light reflectance value obtained during the 10-second recording was used for the signal amplitude for each current (same as in Figs. 5 and 6), and the results of three trials were averaged. Results measured at the macula, perimacular retina, and optic disc are shown for two monkeys (M1 and M2). Response properties appear to be approximately the same in each region under both dark- and light-adapted conditions. Change in reflectance as a function of the electrical current was sigmoidal; weak responses were recorded at low currents from 100 to 400 μA, stronger and faster rising signals were recorded above 400 μA, and maximum signals were recorded above 600 μA. The threshold of the electrically evoked intrinsic signals might have been lower than 100 μA in each of the three regions, but it was technically difficult to determine the peak value of the signal when the absolute light reflectance changes became smaller than 0.05%. A small difference of signal amplitudes between dark- and light-adapted conditions in the perimacular area can be noted (Fig. 4, middle graphs); however, this difference was negligible in amplitude and threshold when compared with that in the flash-evoked response, in which twofold to fivefold differences in signal amplitude and a 3-log difference in the threshold of flash intensity were observed between dark- and light-adapted conditions.[27]

F4 F5 F6

Effect of Stimulus Duration

We measured the intrinsic signals evoked by different stimulus durations in the dark-adapted condition. Stimulus durations varied from 0.5, 1, 3, 5, and 7 seconds, pulse frequency was 20 Hz, and stimulus current was 500 μA for a pulse duration of 10 msec (Fig. 5). Results of four trials were averaged in the two monkeys (M1 and M2). Response properties seem to be almost the same in each region; the intrinsic signals increased with longer stimulus durations and were almost saturated with durations longer than 7 seconds.

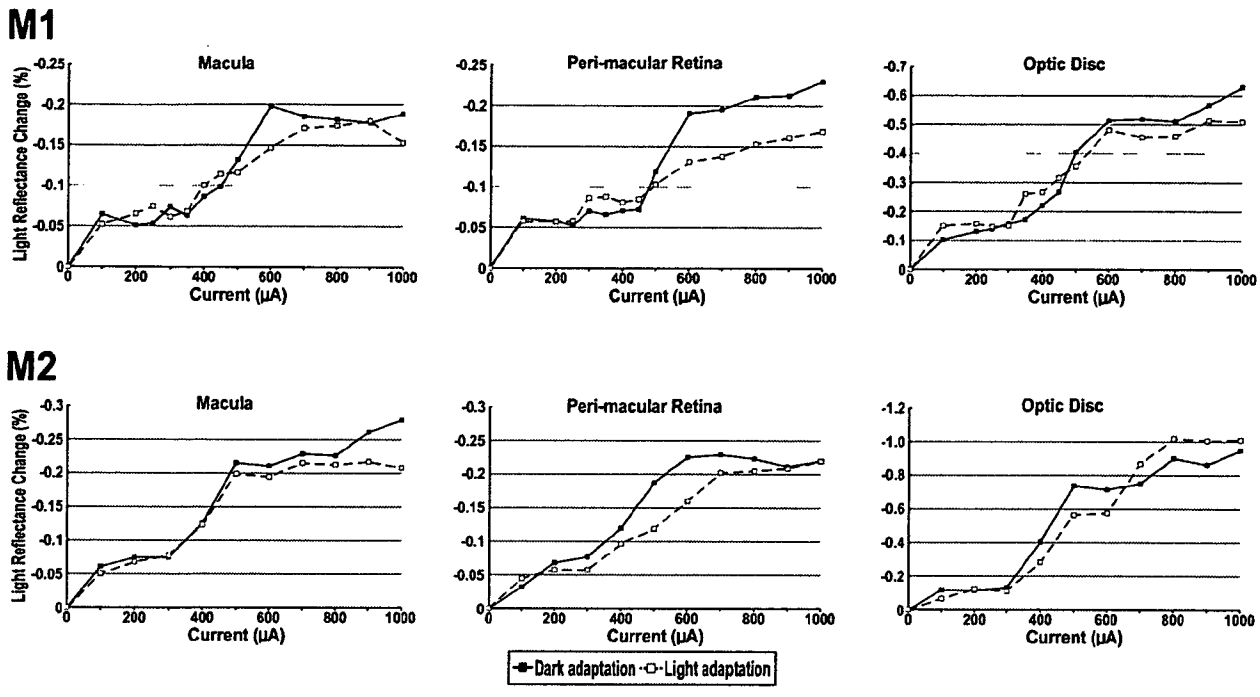

FIGURE 4. Stimulus intensity and intrinsic signals. Changes in the intrinsic signals of three regions after increasing electrical currents (current, 0–1000 μA; total stimulus duration, 1 second; pulse frequency, 20 Hz; pulse duration, 10 msec) in dark- and light-adapted conditions are shown as light reflectance changes in two monkeys (M1 and M2). The peak value of light reflectance decrease during a 10-second recording period was used for the signal amplitude for each current (as in Figs. 5, 6). Note that negative values of light reflectance changes are plotted to indicate the strength of intrinsic signals, and that the vertical scaling is different in three recording regions (as in Figs. 5, 6).

Effect of Stimulus Frequency

We measured the intrinsic signals evoked by different stimulus frequencies under dark-adapted conditions (stimulus current, 500 μA; stimulus duration, 1 second; pulse frequency (Hz)/pulse duration (msec), 5/40, 10/20, 15/13.3, 20/10, 40/5, 60/3.33, 80/2.5, and 100/2; Fig. 6). Results of five trials were averaged for each monkey (M1 and M2).

Response properties seem to have been almost the same in each region; intrinsic signals were maximal when the current frequency was 20 Hz, with one exception in M1 at the perimacular region (15 Hz). The signal was reduced when the frequency was increased or decreased from 20 Hz.

Discussion

Results of this study showed that electrical stimulation through a DTL electrode resulted in a homogeneous change of light reflectance (intrinsic signals) within the vascular arcades of the retina. Unlike the intrinsic signals induced by light stimuli, a peak of the intrinsic signal was not observed at the fovea, and the threshold of the electrically evoked intrinsic signal was not significantly different for the macula, perimacula, and optic disc. In addition, the threshold did not differ under dark- and light-adapted conditions. The strength of the intrinsic signals increased with longer stimulus durations, and maximum signals were obtained when the stimulus frequency was between 15 and 20 Hz.

There are a number of studies, mainly in vitro experiments using isolated retinas, in which the retinal site activated by electrical stimuli was investigated. Results of most of the studies showed that the site activated—e.g., synaptic terminals of the photoreceptor cells,[28–30] bipolar cells,[31–34] horizontal cells,[35,36] amacrine cells,[37] retinal ganglion cells—was more proximal than the photoreceptors.[14,33,34]

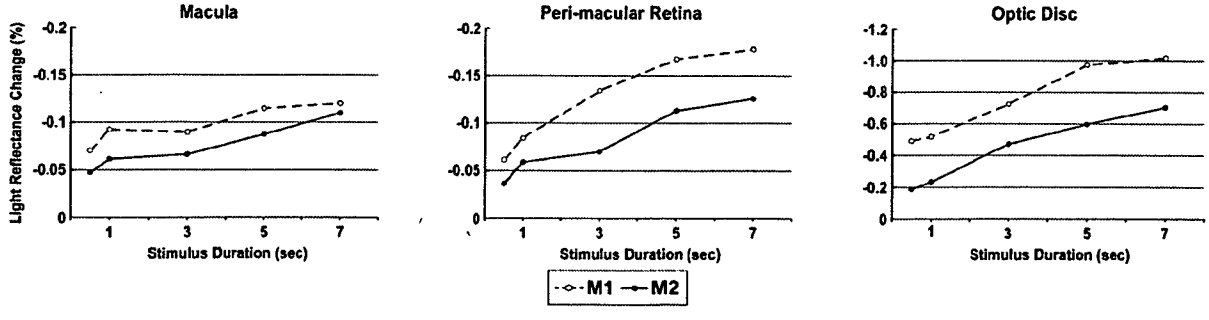

FIGURE 5. Stimulus duration and intrinsic signals. Intrinsic signals of three regions to increasing stimulus durations (total stimulus duration, 0.5 second and 1, 3, 5, and 7 seconds; pulse frequency, 20 Hz; current, 500 μA; pulse duration, 10 msec) in dark-adapted conditions are shown as light reflectance changes for two monkeys (M1 and M2).

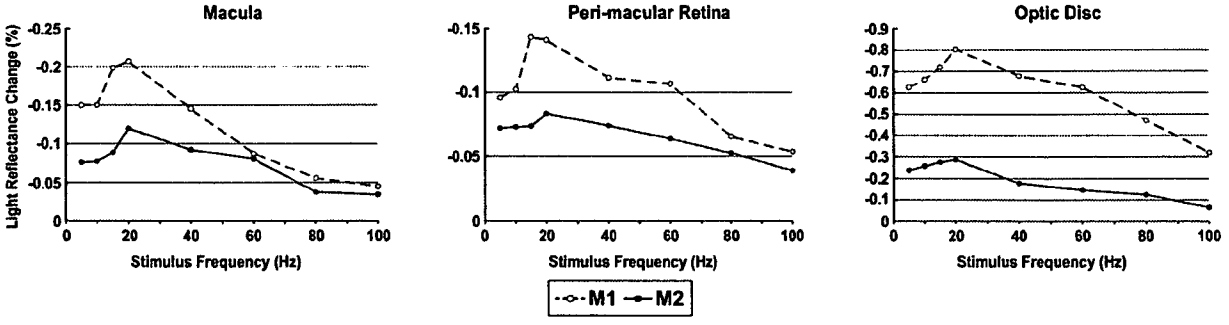

FIGURE 6. Stimulus frequency and intrinsic signals. Intrinsic signals of three regions to increasing stimulus durations (pulse frequency, 5, 10, 15, 20, 40, 60, 80, and 100 Hz; total stimulus duration, 1 second; current, 500 μA) in dark-adapted conditions are shown as light reflectance changes for two monkeys (M1 and M2).

Another method used to identify the site of electrical activation of the retina objectively was the examination of the EER recorded from visual cortex. Thus, Potts et al.[2-4] demonstrated that EER could be recorded in patients with advanced retinitis pigmentosa. They concluded that the site of activation was more central than the photoreceptors.[2-4] Miyake et al.[5-8] showed that the EER is nearly normal in patients with dysfunctional rod or cone visual pathways but that it was extremely abnormal in patients with central artery occlusion. These findings indicate that the retinal origin of EER lies in the middle layer of the retina or close to the retinal ganglion cell layer.[5-8]

The mechanism by which the electrical current is distributed across the retina, however, has not been clearly determined, and the distribution had been estimated mainly by the spatial brightness and extent of phosphenes.[16,38] No study has been reported that estimates the distribution of neural responses over the retina, directly and objectively. In the present study, the current from the DTL electrode enters the eye through the lower anterior part of the sclera and may travel through the vitreous, retina, choroid, or bloodstream to reach the posterior retina. It was not the purpose of this study to investigate the actual pathway of the current. We think a significant amount of the current enters the eye through the sclera and passes through the vitreous body, which also has very low impedance. Brindley[38] designed various types of electrodes that were placed on various locations in the bulbar conjunctiva to investigate the current distribution in the eye by carefully examining the strength and extent of the phosphenes evoked by these electrodes. He concluded that all the electrical phosphenes obtained under the wide range of conditions of his experiments were due to stimulation of the retina by currents flowing perpendicularly to its surface (radial currents through the vitreous humor).[38] Moreover, by observing that the phosphenes were lost as early as 40 seconds from the onset of firm pressure to blind the eye, he concluded that the electrical phosphenes did not result from stimulation of the optic nerve fibers.[38]

When the electrical current is applied from the inferior sclera, one would expect the gradient of stimulation to vary from the inferior retina to the superior retina. Although the current, which spreads radially through the vitreous humor, may not be distributed over the retina in a homogeneous way, the recording region in which quantitative analysis can be reliably conducted is limited to the central 25° in diameter. Thus, we could not measure differences in the signal distribution between the superior and inferior retina outside the vascular arcade.

The intrinsic retinal signals evoked by light stimuli are composed of several components with different properties.[27] Although the precise cellular mechanisms of signal production have not been determined, it is generally believed that the fast signals in the posterior retina (peak time, approximately 150-200 msec) reflect the light-scattering changes after activation of neurons in the outer retina and that the slow signals observed at the posterior retina and the optic disc (peak time, approximately 5-6 seconds) reflect changes in blood flow after neural activation of the cells in the middle or inner layer of the retina. In the later phase, the focally stimulated region showed a focal decrease in light reflectance, with the region corresponding to the location of the stimuli.[27] These findings indicate that the slow components of the intrinsic signals measured in the posterior retina may have a spatial resolution fine enough to indicate the local region of inner retina and can be used for mapping dysfunctional regions due to, for example, glaucoma. AQ: 3

Recently, we showed that the time course of the slow components was strongly correlated with that of blood flow changes measured by laser Doppler flowmetry and that the signals are strongly suppressed by TTX injection into the vitreous cavity, indicating that the slow component of the intrinsic signal are predominantly derived from the stimulus-evoked blood flow increase, which is triggered by the inner retinal activities (Hanazono G, et al. *IOVS* 2007;48:ARVO E-Abstract 528).

In a series of experiments, we have found some discrepancies between the properties of light-evoked and electrically evoked intrinsic signals. First, in the electrically evoked signals, the fast components, which are thought to reflect outer retinal activities, were not observed in the macular and perimacular regions; only slow components were observed (Fig. 2B). Second, the peak of the intrinsic signals in the foveal region evoked by light flashes, which is thought to reflect the activation of foveal cone photoreceptors, could not be observed in the electrically evoked signals, and the response topography in the posterior retina seemed almost homogeneous under dark- and light-adapted conditions (Fig. 3). These findings indicate that the electrical stimuli applied transsclerally do not affect the outer segments of the photoreceptors. We thus believe that the homogenous appearance of the electrically evoked signal may primarily reflect changes induced by the activation of neurons in the inner or middle retinal layers. The most plausible source of the signal is a change in blood flow in the capillaries after activation of the neural cells, although there may be some other cellular mechanisms that can change the light reflectance after electrical stimulation.

When the relationship between the electrical current and the intrinsic signal intensity was examined, we found the response properties seemed to be almost the same under dark- and light-adapted conditions. This is consistent with the previous findings by Miyake,[5] who showed that the amplitude of the EER in humans did not change under dark- and light-adapted conditions. The perceived phosphenes were not altered by the

state of adaptation, and the results of a recent study showed that the threshold of phosphenes is even lower under light-adapted conditions.[16] Taken together with our results, electrical stimulation seems not to be altered by the phototransduction process in the outer segment of photoreceptors.

The relationship between the electrical current and the intrinsic signal intensity was similar in different retinal regions. Under dark- and light-adapted conditions, changes in the intrinsic signal intensity as a function of the electric current were sigmoidal for the three regions studied, and neither the current threshold nor the current giving the maximum intrinsic signal was significantly different. This was, however, not true for the relationship between light intensity and intrinsic signal intensity.[27] In the experiments with light stimuli, the thresholds of intrinsic signals were different, depending on the location of measurement, and the graphs obtained in different regions were completely different. Moreover, there was a shift in threshold to the higher flash intensity to the right after light adaptation. With electrical stimulation, however, the graphs obtained in the three retinal regions were similar and resembled those from the optic disc evoked by light stimulation. This indicates that the electrically evoked intrinsic signals in three regions are related to the blood flow increase after stimulation, though there may be some other mechanisms to induce these signals that are unrelated to blood flow.

With changes in the stimulus frequency of the electrical pulses, the maximal signals were obtained when the current frequency was 20 Hz regardless of the recording region in the ocular fundus. Toi et al.[39] presented an achromatic checkerboard pattern to anesthetized cats and found that the stimulus-related blood flow increase measured by laser Doppler flowmetry was maximum when the stimulus frequency was 20 Hz. The blood flow increase at the optic nerve head after diffuse luminance flicker had physiological properties similar to those of magnocellular retinal ganglion cell neural activities.[40,41] Based on this idea, Riva et al.[42] measured the blood flow increase after 15-Hz flicker stimulus in patients with ocular hypertension and early glaucoma and found that the flicker-evoked blood flow change was abnormally reduced in these patients. These studies suggest a potential in our imaging system to map the dysfunctional regions of the inner retina, such as Bjerrum scotoma in patients with glaucoma. Interestingly, psychophysical studies using flickering stimuli,[43] electrical phosphene,[44] visually evoked potentials,[45] and electrically evoked pupillary reflexes[46] show maximal sensitivities or responses at a frequency of 15 to 20 Hz. The frequency-to-response curves in these studies are similar to those in our study, though the actual sites that regulate this response property are unknown.

In our recording protocol, as the frequency was increased from 5 to 100 Hz, the pulse duration was decreased from 40 to 2 msec, respectively, to keep the total current constant (Fig. 1, inset). There is, however, an in vitro study using isolated salamander retinas, that indicates that the pulse duration is an important factor by which the targeted layer of retina can be determined.[34] The effect of changes in pulse duration in our recording protocol might have influenced the depth of current propagation to some extent.

The resolution of the intrinsic signal topography evoked by electrical stimulation appears to be worse than that evoked by flash stimuli because of the smaller signal amplitudes in the posterior retina. Another factor that might deteriorate the quality of data is the artifacts induced by the electrical current. In a preliminary experiment, we found that currents greater than 1000 μA produce significant artifacts that appear as a mosaic pattern in the posterior pole, possibly because of the muscular contraction of the choroidal arteries by the electrical currents. We found that intrinsic signals could be recorded by transcorneal electrical stimulation by a Burian-Allen contact lens electrode but that the image quality was worse than with transscleral electrical stimulation. This is because the electrical current vibrates the corneal epithelium or the tear film on the cornea, which deteriorates the fundus image observed through the cornea. In our present experimental protocol, we applied the current transsclerally, and it was set lower than 1000 μA to reduce the artifacts.

In conclusion, the results of intrinsic signal imaging indicated that transscleral electrical stimulation is distributed homogeneously over the ocular fundus and represents the activities of neurons mainly in the inner or middle layer of the retina. With further modification of the stimulus protocol and the recording apparatus, it should be possible to record the electrically evoked intrinsic signals in patients. This functional measurement may have potential as a new diagnostic tool for mapping the lesion site of the inner retinal activity, such as Bjerrum scotoma in a patient with glaucoma.

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