Asymmetric visually evoked potentials in human albinos: evidence for visual system anomalies

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Behavioral, anatomic, and electrophysiologic studies indicate that the nondecussated optic system is functionally incompetent and anatomically disorganized in albino animals. The number of nondecussated optic nerve fibers from the retina to the lateral geniculate nuclei, pretectal nuclei, and superior colliculi is significantly reduced in albino mammals, and the laminae of the dorsal lateral geniculate nucleus are abnormal. Visually evoked potentials were recorded from both hemispheres of human albinos of four different genotypes and from normally pigmented human beings under conditions of binocular and monocular illumination. There was a significant asymmetry between the evoked potentials recorded from each hemisphere of monocularly illuminated human albinos, as compared to normally pigmented human beings. The data suggest that there is a disorganization of the nondecussated optic fibers in the human albino similar to that observed in other albino mammals. The abnormalities of the nondecussated optic system are apparently associated with several forms of albinism which result in retinal hypopigmentation.

Key words: albinism, evoked potentials, nystagmus, squint, pigmentation, optic tract, lateral geniculate nucleus.

Vertebrates with laterally placed eyes and panoramic vision usually have complete decussation of optic fibers at the optic chiasm, i.e., in fish, amphibians, reptiles, and birds.4 The proportion of optic fibers which originate in the temporal retinae of mammals increases as the eyes shift to a more frontal position. Along with the development of stereoscopic vision, there is an increase in the proportion of optic fibers that do not decussate at the chiasm. Instead, the fibers project to the optic centers in the hemisphere ipsilateral to the eye of origin (Fig. 1). The proportion of optic fibers that does not decussate varies from approximately 1 per cent in the guinea pig to 10 per cent in the rat, to 20 per cent in the dog and horse, to 30 to 40 per cent in the cat, to 40 to 50 per cent in higher primates.5, 22, 23, 34

For the past 50 years, psychologists have...
used the rat to test whether its relatively small nondecussated optic tract is sufficient for learning of a discrimination between visual patterns. The enucleating or occluding of one eye in combination with the ablating of the striate cortex provides an experimental model that limits visual input to either the decussated or the nondecussated optic fibers. Investigators using the model have reported different results. Chang reported that rats could not learn to discriminate patterns when using only their nondecussated visual system, whereas Muntz and Sutherland reported they could learn the discrimination. In 1965, Sheridan reported that nondecussated optic fibers are functionally incompetent in an albino strain, but not in an ocularly pigmented strain, of the Norwegian rat. It should be observed in retrospect that the above experiments differed with respect to the type of animal used. In each experiment which has tested the functional capabilities of the nondecussated visual system, ocularly pigmented rats learned to discriminate, while the albino rat was found not to learn. Sheridan hypothesized that, "... the paucity of uncrossed fibers that characterizes rodents in general is even further reduced in the albino." Subsequently, Lund verified Sheridan's hypothesis anatomically. Over the next several years, the finding of a reduced nondecussated optic system in albino rats was reconfirmed in behavioral and in electrophysiologic studies.

Giolli and Guthrie's paper on optic projections in rabbits was the first demonstration of a reduced proportion of nondecussated optic fibers in an albino animal other than the rat. In that same year, Guillery reported that the Siamese cat has an unusual nondecussated optic system. Creel subsequently reported that the visual projection area of the Siamese cat is markedly disorganized and pointed out that the Siamese cat is genetically considered to be a mutation at the C or albino locus. More recent morphologic studies, particularly by Guillery and associates, have confirmed the existence of anomalies of the nondecussated optic system of the Siamese cat and of albinos of other mammalian species.

Anatomic studies of several mammalian species have revealed that albino animals have a reduction in the size of the nondecussated retinogeniculate tract and incomplete or abnormal laminae of the dorsal-lateral geniculate nucleus in the albino rat, rabbit, ferret, guinea pig, and
mink, as well as Siamese cat and white tiger.

The paucity of nondecussated optic fibers in albino animals is not limited to the dorsal-lateral geniculate nucleus. There is also an absence of terminations of the nondecussated optic tract in the ventral-lateral geniculate nucleus, pretectal nuclei, and superior colliculus. The finding of abnormalities of the nondecussated optic system in albinos of six species led Creel and Giolli to suggest that the original hypothesis by Sheridan be advanced as a trans-species phenomenon and labeled "Sheridan's Rule." The questions asked in this study are (1) does Sheridan's Rule apply to albino human beings, and (2) does the anomaly, if present, occur only in albinos lacking tyrosinase activity or do other mutations resulting in hypopigmentation of the retina also show the defect?

There are six mutations known in man which result in diseases with features of oculocutaneous albinism, i.e., decreased pigmentation of all of the skin, of the hair and eyes, and decreased visual acuity and varying degrees of nystagmus and photophobia. In addition, two forms of ocular albinism are known in which hypopigmentation of the fundus is associated with nystagmus and photophobia, one form is inherited as an X-linked trait and the other type was observed in members of four families inherited as an autosomal recessive condition (Witkop, unpublished data).

All six forms of oculocutaneous albinism are inherited as autosomal recessive traits, but can be distinguished from each other on the basis of their clinical, biochemical, ultrastructural, and genetic characteristics. Tyrosinase-negative (ty-neg) albinos have a constant phenotype which does not vary with ethnic background; the phenotype is characterized by complete absence of visible pigment. Hair bulbs incubated in L-tyrosine do not form pigment, and melanocytes contain only Stage I and Stage II unpigmented premelanosomes. Tyrosinase-positive (ty-pos) albinos have some visible pigment, although in infancy and in early childhood this may not be clinically apparent in Caucasians. Hair color is usually white-yellow to yellow-brown in color and lightly pigmented nevi may be present. Hair bulb melanocytes may contain early Stage III, lightly pigmented premelanosomes which convert to Stage IV melanosomes when incubated in L-tyrosine. Ty-neg and ty-pos genes are not allelic. Marriages between these two types of albinos produce normally pigmented children which demonstrates that the genes are complementary in the double heterozygote. The yellow mutant (ym albinos) phenotypically resembles the ty-pos albinos but hair bulbs do not form black eumelanin upon incubation with L-tyrosine. When incubated with L-tyrosine plus L-cysteine there is an intensification of the yellow-red color. Melanocytes contain unevenly pigmented Stage III premelanosomes. Hermansky-Pudlak (HP) albinism is a ty-pos type, but in addition has a mild bleeding diathesis due to storage-pool-deficient platelets which lack normal levels of nonmetabolic adenine nucleotides and serotonin and, in addition, have a lipid-storage defect in which ceroid-like material accumulates in reticuloendothelial tissue, in urine, and in the buccal mucosa, in the presence of normal levels of serum vitamin E. Hair bulb melanocytes may contain Stage III pheomelanosomes. Two other diseases with features of oculocutaneous albinism, the Chediak-Higashi disease and the Cross syndrome with microphthalmia, spasticity, mental retardation, and gingival fibromatosis, have been described elsewhere.

In general, there is an increase in amount of pigment and in acuity of vision and a decrease in nystagmus and photophobia through the series of ty-neg → ty-pos → ym → HP.

Due to the difficulty of obtaining anatomic materials from human albinos, it is not feasible to approach the question of visual system anomalies in man from an anatomic point of view. However, it has
been demonstrated that evoked potentials recorded from the visual area of albino rats, guinea pigs, and Siamese cats reflect the anatomically verified reduction and disorganization of nondecussated optic fibers. The evoked potential technique therefore provides a method for evaluating visual system anomalies in man. By covering an eye so that only one eye is photically stimulated, and at the same time recording visually evoked potentials from the scalp overlying visual areas of both hemispheres (at the locations indicated by O1 and O2 in Fig. 1), then relative contribution of optic projections from the nondecussated and decussated optic fibers may be compared. If there is a significant asymmetry between the evoked potentials recorded from each hemisphere of monocularly illuminated human albinos, as compared to normally pigmented human beings, the asymmetry would constitute confirmation that Sheridan's Rule quite probably applies to man.

**Methods**

The participants in the experiments included 9 ty-pos, 8 ty-neg, 2 ym, and 1 HP albino. Sixteen of the albinos were between ages 17 to 32 years; those outside of this age range were two males ages 51 and 56 years, and two females ages 7 and 13 years. Ten normally pigmented individuals with brown eyes, ages 18 to 50 years, participated as control subjects. All albino participants were examined ophthalmologically. None demonstrated ocular pathology with the exception of reduced pigmentation, nystagmus, and refractive error. To date, detailed mappings of the visual fields of 10 of these albinos have been obtained, none exhibited defects of the visual field.

Each subject was seated in a padded chair in a sound-attenuated room. The head was carefully measured and marked to indicate the placement of electrodes based upon the 10-20 international system. Silver disc electrodes were attached bilaterally to the scalp with nonflexible collodion at O1, O2, A1, A2, and Cz. Electrodes were repositioned if there was a measurable asymmetry in the location of any electrode. The resistance of all electrodes was adjusted down to 2 to 5,000 ohm range when the conducting paste was inserted. Resistance and impedance measures were carefully checked between each pair of electrodes to insure an equitable match. An electrode to connect each subject to ground was attached to the scalp anterior to Cz.

A white plastic diffusing screen one meter square was placed 70 cm. from the subject's eyes. The lamp of a Grass PS2C photostimulator, which was enclosed in polyethylene and then packed in rubber foam to attenuate sounds, was positioned 30 cm. behind the diffusing screen. The calculated flash illuminance, which was estimated from measurements by a Minolta flash meter with a micro-disk 2H receptor placed at the location of the subject's eyes, was approximately 2.5 lux. This flash meter measures the maximum illumination in the time period from 10 usec to 33 msec. following activation of the photostimulator.

The subjects were not dark-adapted, although the room was dark during testing. Five sets of 100 flashes, at a rate of one flash approximately every two seconds, were presented to determine the wave form and amplitudinal consistency of each subject's evoked potentials using binocular stimulation. Each subject was asked to count the number of flashes as a control for level of attention. The inked record of the electroencephalogram (EEG) was monitored in an adjacent room to prevent presentation of photic stimuli during periods when artifact was present. There was a rest period of approximately three minutes between each set of 100 flashes.

One eye was then covered with several layers of opaque material consisting of a sterile gauze pad, an opaque black eye patch, and a fitted piece of opaque black paper which covered facial areas around the eye. The head was then wrapped with an elastic bandage, leaving only the uncovered eye, nostrils, and mouth exposed. The efficiency of the occlusion process was checked for each subject. Each subject was rewrapped until no light was perceived via the covered eye. Laterality of occlusion was randomized across subjects.

Brain activity was amplified by a 16-channel Grass Model 78B EEG and recorded on a Mnemotron FM tape recorder. The frequency-response limits of the recording system were 0.01 Hz. and 625 Hz., and the response was linear to 100 Hz. Evoked potentials were recorded from electrodes O1-A1 and O2-A2 for all subjects. Evoked potentials were also recorded from O1-C1, O2-C2, O1-O2, and O2-O1 for some subjects. Care was taken that channel-to-channel bias in the calibration of the EEG did not affect recording of amplitudinal differences of evoked potentials between hemispheres. The assignment of electrodes to channels was varied among the first six EEG channels. Just prior to each daily session the EEG was calibrated.

Electrical responses were averaged by an Enhancetro signal averager that was set to analyze electrocortical activity for 250 msec. following each
flash of light. The averaged response to 100 flashes was plotted by a Moseley X-Y plotter. In addition to visual inspection, statistical comparisons were made between recordings of evoked potentials from each hemisphere by calculating the Pearson product-moment coefficient of correlation during the first 125 msec. of the evoked potentials. In brief, a horizontal baseline for each evoked potential was established on the plot just below the largest positive component, and equally spaced ordinates (1 mm. apart) were projected vertically from this baseline to intersect the plotted evoked potential. Amplitude measures (in millimeters) from the baseline to the point of intersection was calculated for the first 125 msec. These 50 amplitude measures describe the rise and fall of each component of the evoked potential appearing in this time epoch. These 50 numbers, all positive, were correlated with 50 numbers from the evoked potential recorded from the other hemisphere. Interhemispheric coefficients of correlation ranged from 0.80 to 0.99 with a *z*-transformed mean of 0.95 for the monocularly evoked potentials of brown-eyed control subjects. The mean interhemispheric coefficient of correlation for the 20 albinos was 0.70 for the monocularly evoked potentials, which differed reliably from the 10 control subjects (*t* = 4.462, df = 28, *p* < 0.001). The evoked potentials of 14 of these 20 albinos recorded during monocular illumination showed hemispheric asymmetry, whereas monocularly evoked potentials from six albinos showed no significant interhemispheric asymmetry, producing correlations in the same range as the control group. In 10 of the 20 albinos, the potentials evoked by monocular illumination showed extreme asymmetry between hemispheres. One or more components of the evoked potential was missing or significantly attenuated when recorded from the hemisphere that received nondecussated (ND) optic fibers (Fig. 3) and correlations ranged from 0.12 to 0.61. The mean interhemispheric coefficient of correlation for the monocularly evoked potentials of these ten albinos was 0.49 which differed reliably from the correlations of the control group (*t* = 5.755, df = 18, *p* < 0.001). The components that appear in the first 100 msec, in the albinos' evoked potentials were most affected in the hemisphere which receives nondecussated (ND) optic fibers. The nondecussated evoked potentials of the other four albinos also showed a reliable difference as compared to that of brown-eyed control subjects, correlations ranged from 0.38 to 0.67. No hemispheric differences were assignable to sex of the subject or
Fig. 2. Visually evoked potentials recorded from both hemispheres (O2-A1 and O2-A2) of four brown-eyed subjects under conditions of binocular and monocular illumination. Monocularly evoked potentials recorded from the hemisphere receiving the decussated optic fibers (D) can be directly compared to evoked potentials recorded from the hemispheres receiving nondecussated optic fibers (ND). Horizontal time base is 250 milliseconds. Negative is up.
Fig. 3. Visually evoked potentials recorded from both hemispheres (O₁-A₁ and O₂-A₂) of four albino subjects under conditions of binocular and monocular illumination. Monocularly evoked potentials recorded from the hemisphere receiving the decussated optic fibres (D) can be directly compared to evoked potentials recorded from the hemispheres receiving nondecussated optic fibers (ND). Components missing from the ND evoked potential are indicated by dotted lines. Horizontal time base is 250 milliseconds. Negative is up.
laterality of occlusion. Among the 14 albinos with significant interhemispheric asymmetry, there were 6 ty-neg, 7 ty-pos, and one HP albino. Two ty-neg, two ty-pos, and two ym albinos did not show a significant asymmetry.

Discussion

The bilaterally asymmetric, photically evoked potentials of monocularly illuminated human albinos suggest that there is a disorganization of the nondecussated optic fibers similar to that reported for other albino mammals. There was a marked asymmetry in 70 per cent (14) of the albino subjects, but none in the control subjects.

In normally pigmented adults, binocularly evoked potentials are quite symmetrical between hemispheres and usually have coefficients of correlation greater than 0.90.11 The monocularly occluded brown-eyed control subjects in our study, as expected, showed little asymmetry. Even when one eye is enucleated in a human being the hemispheric asymmetry is usually minimal, the difference being a reduction in amplitude of 15 to 20 per cent but with all components usually present.7 However, monocularly evoked potentials recorded from both hemispheres of human albinos not only showed a dramatic diminution in amplitude, but often also the complete absence of one or more components that normally appear during the first 125 msec. after photic stimulation (Fig. 3).

The degree of asymmetry of monocularly evoked potentials from half the albinos was greater than that observed in normally pigmented men with one eye enucleated4 and for most of these albinos was as severe as that which is seen in neurologic patients with heminopsia and/or lesions of the visual pathways.6 Although 10 of the albinos showed dramatic hemispheric asymmetry, and four of them some asymmetry, six of the albinos did not exhibit asymmetrical potentials. The variability among the monocularly evoked potentials of human albinos is not surprising, as there are several sources of variability. First, there is variation in the structure of the laminae of the dorsal-lateral geniculate in man in general.14 Second, it was demonstrated in a sample of an inbred strain of albino guinea pigs, that in two out of six albinos, the laminae of the lateral geniculate resembled that of the pigmented rather than that of albino guinea pigs.8 Among human albinos, there is probably considerable variation in the proportion of nondecussated fibers, in the structure of the geniculate laminae, and in the proportion of nondecussated fibers that terminate in the visual centers other than the geniculate nuclei.

Secondary to the disorganization of retinogeniculate projections is the possibility that asymmetrical evoked potentials are due to disorganization of cortical projections, perhaps similar to the disorganization reported for the Siamese cat.5, 17 The missing components in the nondecussated evoked potential are possibly the result of disorganized geniculostrate projections generating potentials in differently oriented areas of the visual cortex. The visual system anomaly of the nondecussated optic fibers does not appear to be analogous to a lesion of the temporal portion of the retina, but instead a problem of misrouting of these fibers and disorganized projections. The observation that there were no defects of the visual field of the albinos tested is evidence that misrouting, and not reduced numbers or lesion-like impairment of the retina, is possibly responsible for the asymmetrical evoked potentials in human albinos.

Ontologically there is a chronological parallel in the migration from the neural crest of pigment cells and nerves, and in the formation of the optic system. The anomalous structure of the dorsolateral geniculate laminae and subsequent retinotopic representation in the cortex (as well as the reduced number of nondecussating optic fibers) of albino mammals may be occasioned by a chronological sequence of deficits in ontologic development.23 There is evidence from evoked potential studies25
that the development of the nondecussated optic system follows the rule of "ontogeny recapitulates phylogeny," i.e., the nondecussated optic fibers develop, at least functionally, after the phylogenetically older decussated system. Perhaps in the ontogenesis of the nervous system there is a vulnerability in the late developing optic projections which mainly include those related to stereoscopic vision.

The finding that nondecussated optic fibers which terminate in the superior colliculi and pretectal nuclei are usually greatly reduced in number in albino strains of rats, rabbits, guinea pigs, and Siamese cats is of particular interest. Reduction of the proportion of nondecussated fibers that terminates in the midbrain could directly contribute to disturbances in ocular movement by reducing the effectiveness of reflex pathways. The squint commonly seen in albino mammals possibly arises from a deficit of nondecussating projections of the neuronal pool of oculomotor nuclei. Cool and Crawford concluded from their studies of the Siamese cat that squint is primary and not due to lack of binocular coding in the cortex. The evidence that there is a general paucity of nondecussated fibers that terminate in midbrain nuclei when considered along with the evidence that malfunction of the pretectum and superior colliculus affects nystagmus, leads us to speculate that a reduction of functional terminations by nondecussated fibers in the midbrain may contribute to squint and to nystagmus in albinos.

The finding of defects of the optic tract in a variety of animals, including the evidence presented here for a similar defect in man, supports the hypothesis that the optic abnormality is not dependent upon species but is associated with albinism in general. The animals which have, to date, shown evidence of oculonuclear defects have been mutations at the C locus which controls the enzyme tyrosinase, i.e., guinea pig, rat, rabbit, mink, ferret, Himalayan (cch cch) or Siamese (cch cch) cat, and chinchilla (cch cch) tiger. In addition, males of the flecked mouse in which the C locus has been translocated to the X-chromosome, giving a clonal mosaic Lyon effect in heterozygous females, show a reduction in nondecussated fibers.

Oculocutaneous albinism in man is not a single genetic entity. Tyrosinase positive and tyrosinase negative albinism are nonallelic since matings of ty-pos and ty-neg albinos have only produced normally pigmented children. It is not known if yellow albinism is allelic with any other form of albinism in man. The HP-albinism has tyrosinase activity, storage-pool deficiency of platelets, and accumulation of a ceroid-like lipid which suggests on clinical grounds only that it is not allelic with either ty-pos or ty-neg mutations.

Optic neurons of normally pigmented animals do not contain melanin. The question arises as to whether the defect of the optic tract results from an altered extrapigmentary function of the enzyme, tyrosinase, or whether the defect occurs in any mutation which results in hypopigmentation of the fundus. The finding of bilaterally asymmetrical visual-evoked potentials in ty-neg, ty-pos, and possibly HP albinos lends support to the hypothesis that abnormal visual pathways are associated with retinal hypopigmentation and are not the result of an extrapigmentary function of the enzyme, tyrosinase. Further support for this concept comes from work in progress by Witkop's group on pink eye (bbpp) mice, a non-C locus mutant, which have the optic tract defect, and by Guillery and associates of non-C locus pigment mutation in mink. Mutations resulting in fundal hypopigmentation in mink such as pearl, sapphire, and green-eyed pastel, among others, are also associated with defective optic systems.

The basis for the correlation of reduced fundal pigmentation with disorganization of the nondecussating optic system is still unclear. It is tempting to speculate that an intact pigmental system in the develop-
ing optic cup may be necessary to induce normal formation of the nondecussated system. Exceptions to the relationship between albinism and anomalies of the nondecussated optic system may exist, however, in view of the report by Westenberg and Giolli\textsuperscript{11} that they were unable to demonstrate a defective optic system in albino mice of the C57B1/6J strain. This one exception notwithstanding, it would appear that most mammals obey Sheridan's Rule: albino mammals have a paucity of nondecussated optic fibers.

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