wave and used to isolate P III) is readily reversible, that of lack of K is not. Impairment of function by excess K suggests a membrane effect; the minimum requirement points to its participation in some other limiting reaction, examples of which are the kinase reactions catalyzing the initial phosphorylations of glucose. The recording in Fig. 3*a* shows that a threefold increase in K clearly diminishes the *b*-wave, but it unmasks a subsequent positivity and even increases the optical change, suggesting the relief from a bottleneck above the system analyzed. (ii) Succinate as a substrate in some biochemical systems is known to bypass DPN and feed in electrons at a later stage of the respiratory chain with reduced yield in energy, part of which can be utilized to reduce DPN (*10*). On replacing glucose in the medium by succinate, much of the electrical response is maintained. The optical response, however, is completely lost and eventually reverses its sign, as if the system under study were driven backwards. These effects, too, are completely reversible (Fig. 3*b*). On the contrary, the resting absorption decreased markedly on adding succinate, with a concomitant change in the resting electrical potential difference. The transitory absorption increase on stimulation thus seems to require and trap energy when available. (iii) A frequently used means of enhancing spectroscopic effects in respiratory carriers is the addition of nonlimiting concentrations of azide, which slows down the flow of electrons just before they reach the oxygen (*11*). In the ERG, azide has been shown to augment the *c*-wave (*12*). Figure 3*c* shows the combined effects.

On the basis of these results I feel justified in tentatively identifying the stimulus-induced absorption changes as cyclic oxidations of pyridine nucleotides that parallel slow-wave activity of the retina. Specifically, it is the component P I of the ERG that most closely reflects the respiratory processes. This has been found in their quantitative correlation on graded stimulation, in their summating properties (Fig. 2*b*) under limiting (Fig. 3*a*) as well as facilitating (Fig. 3*c*) conditions, and can be demonstrated in their simultaneous exhaustion (Fig. 3*d*). The determining role on retinal excitability of slow electrical processes has long been recognized (*7*) and has recently been demonstrated in the isolated human retina (*13*). It seems substantiated by the measurements reported here.

This work appears to introduce knowledge on respiratory control mechanisms for the interpretation of bioelectrical slow-wave activity and demonstrates the manifestation in this preparation of oxidation-reduction states in measurable potential differences.

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# Electroretinogram in Newborn Human Infants

Abstract. *The electroretinogram of the newborn human shows the* x-*wave component which was demonstrated by Adrian and others to be a concomitant of photopic visual function in the adult. This finding may provide electrophysiological support for behavioral observations indicating that infants have some color vision and ability to resolve visual stimuli.*

Evidence has accumulated from several recent studies which suggests that the human visual system is more highly developed at birth than was once realized. In 1962 Fantz, Ordy, and Udelf (*1*) reported that infants under 1 month of age fix their eyes upon stripes subtending a visual angle of about 40 minutes of arc. Their results correspond roughly to visual acuity ratings in the newborn obtained by Gorman, Cogan, and Gellis (*2*) in their study of opticokinetic responses. Recent work by Dayton and his co-workers (*3*) indicates that some newborns show a well-developed fixation reflex and can resolve stripes subtending only 7.5 minutes of visual angle, the equivalent in Snellen notation of 20/150 vision. As early as 1932, Staples (*4*) demonstrated that 3-month-old infants tend to look at colored rather than gray papers equal in brightness. Also, Berlyne (*5*) and Fantz (*6*) have observed that infants fixate patterned stimuli longer than homogeneous fields.

Early work with the electroretinogram (ERG) of human infants has not confirmed the degree of visual development indicated by these behavioral studies. Zetterström (*7*) was the first to find electroretinograms in human infants. She reported scotopic *b*-waves of long latency and very low amplitude appearing within the first 3 days of life in full-term infants. By 8 weeks of age the waveforms and latencies became comparable to those of adults. Combined data on premature and full-term infants showed a positive correlation between birth weight and appearance of the ERG (*8*). In contrast to this, Horsten and Winkelman (*9*) found that both negative *a*-waves and positive *b*-waves of low amplitude but with adult characteristics were present in both premature and full-term infants when stimuli of sufficient intensity were used. They succeeded, moreover, in obtaining ERG responses at rates of stimulation as high as 72 flashes per second. Since the response which appears at this rate is photopic in the adult (*10*), their work suggests that the infant may have photopic capabilities.

In the study reported here, our aim was to demonstrate the *x*-wave in the electroretinograms of newborn humans. This wave, when elicited by stimulation with light of long wavelength, was shown by Adrian (*11*) and others to be clearly related to photopic vision. Although the *x*-wave is (as all components of the electroretinogram are) generally recognized as a mass retinal action potential which cannot reflect developmental aspects of visual acuity or pattern perception directly, it may be possible to interpret it as an index of the maturation of the photopic mechanisms. Thus, presence of an *x*-wave strengthens the interpretations which have been made of previous behavioral and electrophysiological experiments.

Twenty recordings were made, and of these, 15 records obtained from 12 infants were complete and technically satisfactory; the 15 records form the basis of this report. The infants consisted of seven males and five females ranging in age from 6 hours to 5 days. They were born to mothers who had received minimal or no analgesic agents during labor and who had not had general anesthesia during delivery.

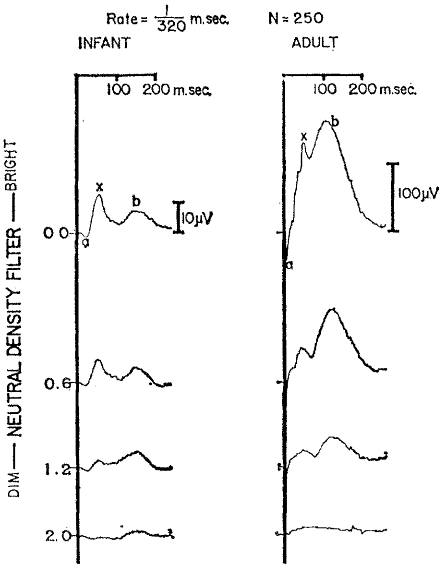

Fig. 1. Averaged ERG responses to orange flashes (one flash per 320 msec) at different intensities for a sleeping 64-hour-old infant compared with those of an adult with eyes closed. The infant's stimulated eye was held open by a speculum; however, the pupil was partially rolled under the upper lid. Upward deflection denotes positivity of the cornea. At 0.0 on the ordinate, no neutral density filters were in the optical path—that is, the maximum stimulus. Filters decreased the luminance in the log-unit steps indicated.

The methods used for recording were similar to those used for the study of the adult visual system, with some modifications necessary because of special problems encountered with infants. Recording was performed in an especially constructed two-level booth. Recording amplifiers were mounted at the lower level while the electrically shielded upper level of the unit contained the infant's bassinet. A headholder was used to keep the head in midline, and the arms were swaddled so that the infant's hands could not reach its face.

The ERG was recorded with a contact lens electrode which was modified from the Riggs design by Grant (*12*). A specially designed speculum which fitted under the eyelids and held them apart during recording was attached to the lens. A viscous contact lens solution was used which effectively prevented the appearance of artifact-producing bubbles under the contact lens. The reference electrode was on the right mastoid.

Since the electroretinogram of infants is small, slowly flickering stimuli (three flashes per second) were used and the recurrent responses were averaged ($N = 250$) with a Mnemotron computer of average transients. Responses to a series in which the stimulus was of long wavelength and was applied at several intensities, were recorded and compared with responses to a similar series of white flashes.

The stimuli were provided by a Grass PS-1 photic stimulator mounted 26 cm above the infant's eyes and masked except for a circular opening 3.7 cm in diameter which subtended a visual angle of 8°. It was always operated at its maximum output; luminance was varied by the use of neutral density filters. Measurement of the illumination provided by the stimulus presented a technically difficult problem. An approximate specification of the stimulator output was made by matching it to a steady illumination. When the stimulus flickered at a rate of 40 flashes per second with no filters in the light path, the illumination which fell upon the infant's face was equal to a steady white illumination of 64.9 lu/m$^2$. For test conditions requiring orange-colored stimuli, a Corning HR red-shade 34 filter, uniformly transmitting wavelengths above 580 m$\mu$, was placed in the light path.

After the infant was fed, the pupil of one eye was partially dilated with 10 percent Neosynephrine. Before insertion of the lens a drop of a short-acting local anesthetic (proparacaine hydrochloride, 0.5 percent) was put in the eye. After a 15-minute period of dark adaptation, the infant was presented with a fixed sequence of stimuli covering a range of luminances of both orange and white flicker.

In some of the sleeping infants the pupil tended to roll up and under the upper lid, even though the speculum held the lids apart. In such instances the amount of light actually reaching the retina was diminished. The lids probably also acted as a red filter, thus influencing the wavelength reaching the retina as well as the luminance. Control data for adults with the eyes open and closed were obtained. The

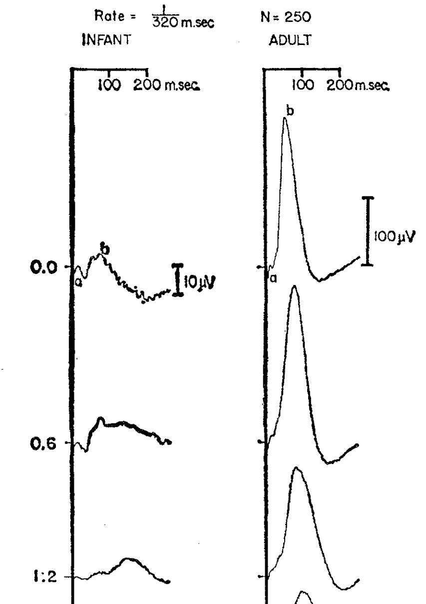

Fig. 2. Averaged ERG responses to white flashes (one flash per 320 msec) at different intensities of a sleeping 6-hour-old infant compared with those of an adult. The infant's stimulated eye was held open with a speculum and the pupil was aligned with the light source; the adult was looking at the stimulus. Upward deflection denotes positivity of the cornea.

closed-eye condition in the adults probably cut off even more light to the retina than the open-eye condition, with the pupil rolled back, in the infant.

The ERG's obtained from the infants were similar in form to those obtained from the adults, but were of lower amplitude. When an infant was stimulated with light of long wavelength, a negative potential occurred soon after the stimulus and was followed by two positive deflections which were comparable in latency to the *x*- and *b*-waves of the ERG's of the adults. The latency of the *b*-wave shifted with changes in intensity, but the latency of the *x*-wave was relatively constant. Additional small ripples occurred in the ERG's of both the adults and infants, but were more marked in those of the infants.

In Fig. 1, the averaged responses obtained from a 64-hour-old infant are compared with those obtained from an adult in response to stimulation with the orange-colored flashes of light. When the orange stimulus of maximum luminance (0.0) was presented, a positive wave with a peak latency of approximately 50 msec occurred and was followed by a second positive wave. The overall amplitude of the ERG response decreased as stimulus intensity diminished, but two positive deflections were usually distinguishable at relatively low levels of luminance. The adult ERG shows an *a*-wave, *x*-wave, and *b*-wave of the sort that have been well described in the literature. These components are reduced in amplitude when stimulus luminance is decreased. Latency of the *b*-wave increases, but *x*-wave latency remains about the same. Under the control condition where activity in the absence of light stimulation was recorded, only low-amplitude fluctuation of the base line was apparent in the averaged ERG potentials of all infant and adult subjects.

In Fig. 2, the ERG responses of a 6-hour-old infant to white flashes of light are compared with those of an adult. The pupil of the infant's stimulated eye appeared well aligned with the light source; the adult was looking directly at the stimulus. This infant's ERG is somewhat more rippled than was typical for the group and shows a definite double positive deflection. As light intensity decreases, the first of these components becomes less conspicuous and the second component increases in latency.

Changes in amplitude of the response with orange and white light and diminishing flash intensity are graphically presented in Fig. 3. The peak-to-peak amplitude of the ERG to white flashes was measured as a description of the *b*-wave and is shown in the left half of the figure. For all of the infants as for the adults, the amplitude of this wave decreased as stimulus luminance was reduced. This relationship is significant beyond the .001 level of probability according to the results of a Spearman rank-order correlation (*13*). When the adult subject closed his eyes, the ERG sensitivity decreased by approximately one log unit, but his response is still much larger than that of any of the infants. In the ERG's of the infants, there was no significant indication of any change with age occurring in the amplitude of the *b*-wave; this might be due to our limited sample and the considerable individual variation found in electrophysiological measures.

The peak-to-peak amplitude of the first positive wave obtained in response to orange light is shown on the right-hand side of Fig. 3. The amplitude of this component is also a function of stimulus intensity, a relationship significant beyond the .001 level of probability when tested by means of the Spearman rank-order correlation. The amplitude of this wave is much smaller in the infant than the adult at all luminances.

There is some suggestion of a tendency for younger infants to have smaller *x*-waves than older ones, but there is again considerable overlap of scores in this small sample.

Mean latency of the peak of the

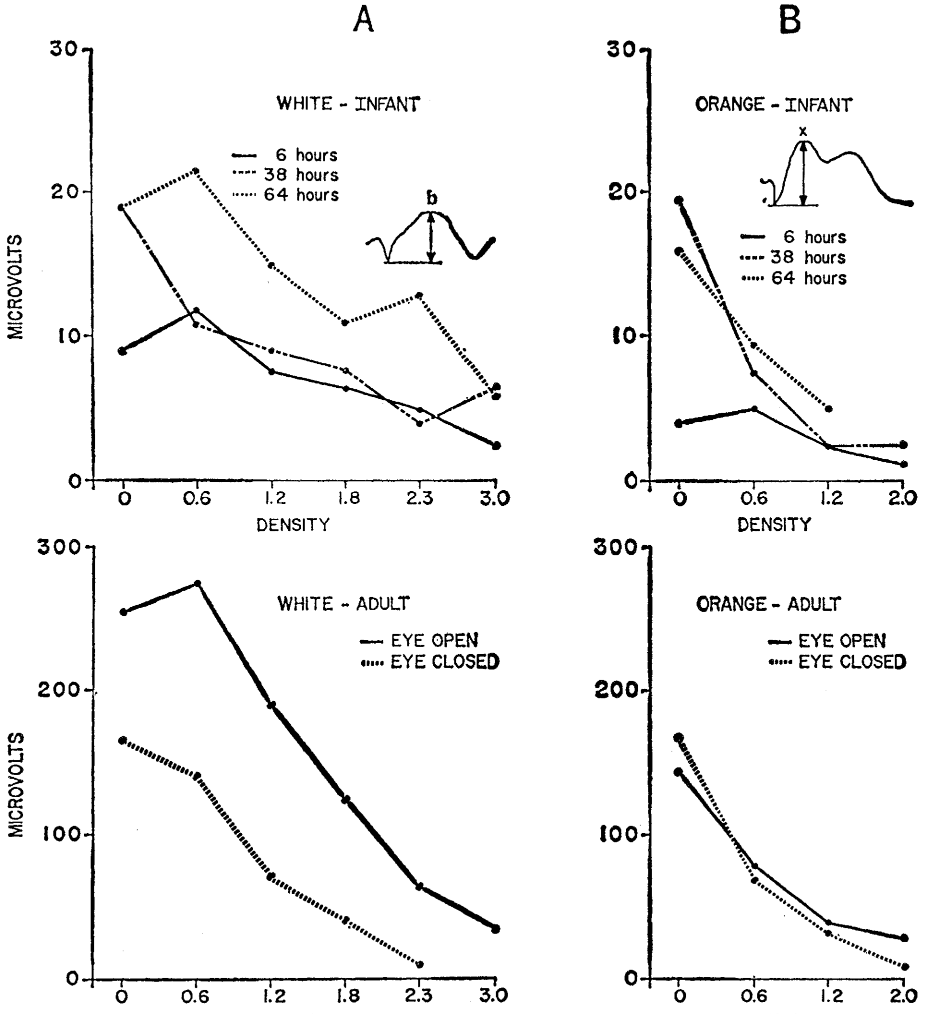

Fig. 3. Averaged *b*- and *x*-wave response amplitudes obtained in response to a series of flashes of white (*A*) or orange (*B*) light of varying intensities. Results in the two upper graphs are for three infants of different ages; results in the two lower graphs are for an adult under both the eye-open and eye-closed conditions. Because the responses of the adult are 10 times larger than those of the infants, a different scale has been used for the ordinate in the lower graphs.

*b*-wave for all of the infants tested was 96 msec with the maximum white stimulus. It increased with decrease of stimulus luminance, a relationship significant beyond the .001 level of probability according to the results of a Spearman rank-order correlation. Mean latency of the infant *x*-wave was 57 msec. It did not change with stimulus luminance. These values are somewhat longer than those seen in adults.

The ERG of the newborn is highly differentiated. The components are similar to those of the adult ERG, but they are attenuated and modified in form. In both adult and infant the response depends not only on the intensity of the stimulus, but also on the wavelength. The electroretinogram was small in the infant; peak latencies of *a*-, *x*- and *b*-waves were slightly longer for the same stimulus luminance and the response seemed to be more rippled. But despite these differences, the wave pattern of the response is remarkably similar to that of adults (*14*). The first positive wave elicited in the infant by stimulation with orange light appears to be the same as the photopic or *x*-wave of the adult ERG because of its form, latency, and relationship to stimulation with red light.

The finding of the *x*-wave in the ERG of infants stimulated with light of long wavelength supports the assumption that photopic vision is functional in the newborn human. The finding of a *b*-wave suggests that the scotopic system also is active. Hence, both systems develop together at the retinal level. Our results may provide some electrophysiological support for behavioral studies of the development of responses to color and form such as those cited earlier.

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## Auditory Habituation: A Test of a Centrifugal and a Peripheral Theory

Abstract. *Evoked potentials recorded at the cochlear nucleus of unanesthetized cats show that auditory habituation is a process with a rapid onset and a slower rate of dissipation, and that the habituation is a direct function of the rate of stimulation. Neither the middle ear muscles nor the reticular formation appear to participate in this process. It is suggested that auditory habituation might be a function of the interaction of the olivocochlear bundle and some intrinsic property of higher synaptic areas.*

A considerable body of evidence (*1*) indicates that some forms of afferent input are under centrifugal control. Studies of habituation of the evoked potentials recorded in the auditory pathway of unanesthetized animals also provide experimental support for this concept by indicating that habituation is under centrifugal control (see *2, 3*).

Another interpretation of auditory habituation which has been proposed is that known as the peripheral theory, according to which auditory habituation is mediated by the activity of the middle ear muscles. Guzmán-Flores (*4*) reports that the cutting of the middle ear muscles prevents habituation and the administration of curare abolishes habituation. Since the effective latency of these muscles (*5*) is far greater than the duration of stimuli usually employed, it is argued that habituation is produced by temporal conditioning of the muscles. The auditory stimulus acts as an unconditioned stimulus, and the interval between stimuli as a conditioned stimulus for the contraction of the muscles. An important implication of this theory is that a constant interval between stimuli is a necessary condition for habituation to occur. Another implication is that any change in this constant interval after habituation has developed should lead to dishabituation or a return to evoked potentials with amplitudes similar to those found before habituation. However, in one study (*6*) it is reported that habituation occurs in the auditory cortex despite bilateral cutting of the middle ear muscles. This observation, while not crucial, is not in strict agreement with the peripheral theory.

According to centrifugal theories, habituation is mediated by direct inhibition from a center in the brain. In one theory this role is allocated to the reticular formation, since barbiturate anesthetics will abolish habituation and extensive lesions in the mesencephalic reticular structures will prevent it (*2*). Habituation is also distinguished from adaptation or some fatigue process, since a change in the habituating stimulus pattern will produce an immediate dishabituation. This effect is regarded as a change in the animal's "attention," which is mediated by the reticular formation.

In another centrifugal theory it is argued that the direct inhibition comes from a cortical locus, but the experimental support for this concept is meager (*3*). According to this theory, a change in the stimulus pattern will produce dishabituation and an associated change in "attention."

The original evidence for the reticular theory was obtained from studies of habituation at the cochlear nucleus, but other workers (*7, 8*) were unable to find significant habituation in this region. However, more recently (*9*), habituation was reported to occur at the cochlear nucleus, but the decrements were considerably smaller than those of the original observations. In view of the conflict in the experimental data relating to both theoretical positions, the experiments reported here were designed to study habituation at the cochlear nucleus and to provide a test of both theories.

Fourteen cats were used. Bipolar electrodes were permanently implanted in the cochlear nucleus at Horsley-Clarke co-ordinates P 7.5, R 8.0, H −5.5 by a method previously reported