from infants two to six months of age using a 15 minutes of arc, as do adults with 20/20 acuity. By six months, infants produced the largest amplitude VEP to checks subtending visual angles of 7.5 or 15 minutes of arc, as do adults with 20/20 acuity.

This finding indicates that by six months an infant's sensory capacity for a visual acuity of 20/20 is established.

When visually evoked potentials (VEP) are recorded from adults using checkerboard pattern stimuli, there is a close correlation between the relative amplitude of the pattern VEP to different check sizes and subjectively determined visual acuity. The largest amplitude VEP in an adult with 20/20 acuity is found with checks subtending 10 to 20 minutes of arc; as the size of the checks is increased or decreased the VEP amplitude is attenuated. In addition, the peak of the adult VEP amplitude-check size function shifts to larger check sizes as the subjects' acuity is degraded with ophthamaline lenses of increasing power. This link between the pattern VEP and subjective acuity in adults can be used to measure infant acuity since there is no accurate way to measure their acuity subjectively. In the present experiment, we have recorded the VEP from infants with a checkerboard pattern reversal stimulus using checks which subtended visual angles of 7.5, 15, and 30 minutes of arc and then compared these data to that obtained from adults with 20/20 acuity. Using checks of less than 30' will more readily assure that the VEP is contrast specific and is elicited from central regions of the retina. In addition, a pattern reversal stimulus maintains a constant state of luminous flux which minimizes luminance contributions to the VEP.

Methods. The VEP was recorded from 15 infants between the ages of two and six months. A single electrode was placed over the inion along the midline and referenced to the right ear. The mother held the infant 75 cm. from the checkerboard stimulus, which subtended a visual angle of 12°. The checkerboard patterns were produced by polaroid vectograph prints and pattern reversal was obtained by rotating a sheet of polaroid between the light source and each of the vectographs. The luminance of the stimulus field was 75 ft. Lamberts. The contrast of the checks was 0.75. One experimenter remained in the shielded room with the mother and infant and had a remote control switch to operate a computer of average transients. When the experimenter was satisfied that the infant was quiet and fixating, the averaging was initiated. The experimenter's criterion for starting the averager was that the reflection of the stimulus field be in the center of the pupil. Since the total mean luminance of the pattern reversal field remains constant, the experimenter sees a continuous reflection from the cornea. This allows for a more reliable judgment of fixation. While we cannot be absolutely certain that our criterion for fixation ensures that the infants had the pattern stimuli in focus on the retina it is clear...
Fig. 1. Tracings of VEP obtained from a four-month-old infant. Records on the right are before filtering; records on the left show the signals after filtering. Stimulus alternation: 12 per second; analysis time: 400 msec.; sweeps accumulated: 32; check size shown on the left. Vertical line, 25 nV; horizontal line 100 msec.

Fig. 2. Amplitude (μV) of pattern reversal for two infants as a function of check size. Open circles, two months; closed circles, three months; open squares, four months; closed squares, five months; open triangles, six months; closed triangles, adult. No data points for 7.5' checks are shown for SB at two months and BR at two and three months because their responses did not differ significantly from a control condition (VEP's recorded when the stimulus light was turned off).

that by two months they have sufficient accommodative power to accomplish this and a strong preference for patterned stimuli. If the infant looked away from the stimulus the averaging was stopped and then restarted when the infant resumed fixation. Signals were led to a standard electroencephalograph (EEG) pre-amplifier with a band pass of one and 35 Hz. and then through an active narrow bandpass filter with a Q (quality) of 10. The Q is a measure of the sharpness of the bandpass of the filter at one center frequency. In the present experiment the center frequency was 12 Hz. and signals at 10 and 14 Hz. were attenuated by 5 db. The filter is active because its center frequency was calibrated to match the stimulus frequency. This “phase-locked” filter prevents any distortion of the VEP's. Fig. 1 shows records obtained from one infant with and without the filter. The narrow bandpass filter increases the signal to noise ratio which allows one to record measurable signals over a shorter time interval.

Results. Fig. 2 shows the amplitude of the pattern reversal VEP as a function of check size for two of the infants in the study and for an adult with a visual acuity of 20/20. Both infants show an increase in the absolute amplitude of the VEP up to five months and then a decrease at
Fig. 3. Relative amplitude of the pattern VEP as a function of check size for infants between the ages of two and six months and six adults. Data points represent the mean value of normalized VEP amplitudes and the vertical lines represent one standard error of the mean.

Table I. Location of the peak of the amplitude check size function for infants at different ages and adults. The Table shows for each age range whether or not a peak amplitude was present and, if so, the check size at which it occurred. We have arbitrarily established the criterion that for a peak to be present, the amplitude of the largest signal must be 2 μV greater than the amplitude of the smallest signal. Also given are the results from six adults who were tested under identical recording conditions and had acuities of 20/20.

<table>
<thead>
<tr>
<th>Age</th>
<th>Location of peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5'</td>
<td>15'</td>
</tr>
<tr>
<td>2 months</td>
<td>0 0</td>
</tr>
<tr>
<td>3 months</td>
<td>0 0</td>
</tr>
<tr>
<td>4 months</td>
<td>0 0</td>
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<tr>
<td>6 months</td>
<td>2 2</td>
</tr>
<tr>
<td>Adult</td>
<td>2 3</td>
</tr>
</tbody>
</table>

*This subject peaked at 15' but did not meet the criterion amplitude difference of 2.0 μV. The subject’s amplitude for 15' checks was 1.5 μV greater than his smallest check-size amplitude.

There was a greater relative increase in the VEP amplitude for 7.5' checks. Subject BR shows a similar trend; a shift in the peak and a small relative difference in amplitude between 7.5' and 15' checks by six months of age.

Table 1 shows how the relative amplitude of the pattern VEP to different check size changes between the ages of two and six months for individual subjects. Initially, the VEP amplitude-check size function is either flat, or peaks at 30'. By three months, 9 out of 10 infants peak at 30'. At four months, four infants peak at 15', five infants peak at 30', and one had no peak. All of the infants tested at five months peak at 15'. By five months the distribution is similar to that found for six adults: some peak at 7.5' and others at 15'.

Fig. 3 shows the mean relative amplitudes and standard error of the mean for all infants and adults. The absolute amplitude data were normalized by converting them to Z scores and the curves show that the peak of the inverted "V" shaped amplitude check size function shifts from 30' to 15' between the ages of two and six months and that the descending slopes of the functions for six-month-old infants and adults with 20/20 acuity are similar.

Discussion. While one is justified in converting psychophysically determined grating thresholds to Snellen values, or their equivalent, one cannot similarly convert the check size of the largest VEP amplitude to analogous Snellen values. All of the adults tested in the present study had...
Snellen acuities of 20/20 and peak VEP amplitudes of either 7.5 or 15 minutes of arc. If these check sizes were converted to Snellen terms, it would indicate visual acuity of 20/150 to 20/300, which is clearly not the case. However, the issue under consideration is at what point does the relative shape of the infant VEP function resemble the adult function. We find this occurs at six months and submit that this similarity of the infant and adult check size function provides indirect evidence that by six months the infant visual system is able to process spatial information as accurately as an adult. Further, the VEP unlike retinoscopy provides a valid and more direct means of measuring acuity since it reflects the electrical activity of the visual system from the photoreceptors to the occipital lobe. We did not perform retinoscopy on the infants since it would have exposed them to the risks involved in the use of cycloplegics. Also, the fact that all of the infants by six months had amplitude check size functions which peaked at 7.5' or 15' and were similar to an adult emmetrope’s function could be viewed as support for the absence of any significant refractive errors in our subjects.

Behavioral studies of infant visual acuity using differential fixation techniques indicate that the best acuity for a six-month-old infant is between 20/70 and 20/100. This does not necessarily indicate that acuity determined by behavioral methods is poorer than electrophysiologically determined acuities since gratings finer than 3.5' minutes of arc (20/70) were not used in these studies. In fact, there is no reason to expect that the electrophysiological and behavioral acuities would agree since the results of behavioral studies depend on aspects of vision which are peripheral to the sensory evoked potentials. Our data do suggest, however, that there is a period of rapid sensory change during the first six months of life which may point toward a more expeditious handling of some infant visual disorders such as ptosis, congenital cataracts, and strabismus. Prevention of normal visual experience during this period by these clinical problems may partially contribute to deprivation amblyopia.

The question of electrophysiologically determined visual acuity in infants younger than six months still remains open. Harter, Deaton, and Odom used a flash elicited checkerboard pattern to measure evoked potentials in 10 infants between the ages of six and 45 days and in one infant between the ages of 21 and 156 days. They found that between the ages of six and 45 days there is a shift in the peak of the amplitude check-size function. Larger amplitude responses were found with checks subtending 11' and 22' of arc for six to nine-day-old infants but by the age of 45 days larger amplitude responses were elicited with checks subtending 180' of arc. After 45 days, there is a second shift back to smaller checks elicit the largest VEP. They conclude that the peak amplitude to small checks in infants younger than 45 days reflects the processing of subcortical mechanisms while the re-emergence of a peak to small checks in the infant older than 45 days reflects the activity of cortical mechanisms. Whether the results found with infants younger than 45 days would also occur with a pattern reversal stimulus remains to be determined since none of our infants were younger than 60 days.

At present, we are recording pattern reversal VEP's from infants between the ages of one and six months using a greater number of check sizes less than 30'; this includes the high spatial frequency side (finer checks) of the inverted V-shaped amplitude-check-size function. Further, we are determining VEP acuity thresholds in these infants by extrapolating the regression line of the amplitude check-size function to zero microvolts, a technique which has been shown to give close agreement with psychophysical thresholds in adults for spatial contrast sensitivity.

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Key words: visually evoked potentials, infant acuity, checkerboard pattern stimuli, pattern reversal stimuli, infant electrophysiology, infant evoked potentials.

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Resting membrane potentials, electrical resistance, and coupling of chick retinal pigment epithelium cells in tissue culture have been measured with micropipette electrodes. Topical application of NaCl, KCl, MgCl₂, and CaCl₂ produce rapid, reversible changes in the membrane potentials of these cells. CaCl₂ uniquely induces a slow hyperpolarizing wave that can lead to barrages of depolarizing action potentials or spikes and a slow, reversible vacuolization of the cells.

Chick retinal pigment epithelium can be maintained and cloned in tissue culture,¹ providing a potentially useful method of examining the functional properties of these cells under high-power microscopic observation and isolated from other elements in the eye. During experiments designed to examine some basic physiologic properties of single cells in these cultures, we noticed an unusual phenomenon produced by Ca²⁺ which we would like to describe.

Single pigment epithelium cells, growing in monolayered colonies (Fig. 1), were penetrated with micropipette electrodes under direct vision using an inverted microscope. The cells were growing in approximately 4 ml of culture media in Falconer plates, 5 cm in diameter. The techniques used to culture these cells have been described elsewhere.¹ The methodology used to record from and stimulate single cells electrically under these conditions resemble those of Nelson, Ruffner, and Nirenberg in their studies of neuroblastoma cells.

Results are based on well differentiated cells growing in the center of mature colonies (Fig. 1); such cells are taller and relatively easier to penetrate than those, growing at the edge of a colony, which are flatter and less differentiated. All cells, cleanly penetrated, had large stable, negative membrane potentials (64 ± 13 mv.; n = 285). The electrical resistance of these cells measured by passing current through the recording micro-electrode and cell in a bridge circuit² was relatively low (35 ± 10 x 10³ ohms; n = 25). Part of this low resistance is due to electrical coupling between cells which was demonstrated in two pairs of cells using two separate micro-electrodes. Electrical coupling between pigment epithelial cells is characteristic of mature, intact retina¹ and its existence in tissue culture adds further evidence to support the highly differentiated state of these cells.

The application of small amounts (5 to 10 µl) of 0.5 M solutions of NaCl, KCl, MgCl₂, or CaCl₂ just above the surface of the culture media and near the cells in question produced rapid, reversible changes in the membrane potentials of these cells (Fig. 2). NaCl, KCl, and MgCl₂ depolarized the cells, the K⁺ salt being more effective than the others (see Table I). CaCl₂ produced an initial depolarization which was followed by a larger hyperpolarizing wave (Fig. 2). Half of the time this hyperpolarization was followed by a barrage of depolarizing action potentials (Fig. 2, inset). These impulses or spikes were rhythmic, all-or-none responses that usually occurred in clusters, sometimes singly but never spontaneously or after the other solutions. Fig. 3 shows an oscillograph of such a single pigment epithelial cell spike.

In addition to this electrical change, a curious, slow, transient vacuolization within these cells was also noticed after application of CaCl₂. This optical change was subtle for it was missed in all the initial experiments. Even after we suspected its presence, it only became obvious after looking at time lapse photographs (Fig. 1). The vacuolization begins at about the time the spikes appear but far outlasts them. It is not yet clear how dependent the vacuoles are on the spike discharge.

The concentration of Ca²⁺ necessary to trigger these events is not easy to measure. The quantity of solution we have used raises the overall con-

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Table 1

<table>
<thead>
<tr>
<th></th>
<th>Depol. (mv.)</th>
<th>Hyperpol. (mv.)</th>
<th>Spikes</th>
<th>Time (sec.)</th>
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<tbody>
<tr>
<td>CaCl₂</td>
<td>26 ± 4</td>
<td>11 ± 5</td>
<td>13</td>
<td>29 ± 17</td>
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<tr>
<td>NaCl</td>
<td>9 ± 4</td>
<td>3 ± 5</td>
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<tr>
<td>MgCl₂</td>
<td>7 ± 3</td>
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<td>0</td>
<td>—</td>
</tr>
<tr>
<td>KCl</td>
<td>8 ± 6</td>
<td>12</td>
<td>0</td>
<td>—</td>
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</table>

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