Visually evoked responses in amblyopia

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Experimental work on kittens has demonstrated that a normal visual environment is required to develop and maintain the special response characteristics of cells in the visual cortex. It is therefore very tempting to extrapolate these findings and explain the defects in human amblyopes by the same abnormalities in the visual environment. However, direct analysis of the function of the visual cortex in patients relies on indirect methods of investigation: because of practical difficulties, refinements of psychophysical testing (Campbell, Kulikowski, and Levinson, 1966; Kulikowski and King-Smith, 1973) have rarely been employed in clinical work. Although, in the analysis of cortical defects, such investigations may highlight the abnormality and enable a precise description of the defect to be given (*e.g.* Bodis-Wollmer, 1972) in psychophysical terms, extrapolation from the psychophysical description to the cellular mechanisms remains very difficult. Another line of approach is to record cortical activity by using evoked potentials. Some studies of cases of amblyopia have been made with such a technique (Spekreijse, Khoe, and van der Tweel, 1972) and in these, as in normal subjects, correlations have been established between evoked potentials and psychophysical sensitivity. Such analogies exist between various perceptual functions and their electrical analogues, *e.g.* binocular vision, suppression of visual acuity and colour vision (see Regan, 1972, for a summary), but in such investigations the stimulus has to be very carefully controlled and its production requires elaborate apparatus while the analogy can be drawn only after prolonged experimentation.

For some years we have been using visually evoked responses (VERs) as an objective test of eye function (Behrman, Nissim, and Arden, 1972), using as a stimulus a pattern-reversing chequerboard. In order to make our test of practical clinical use, the testing procedure and analysis have had to be simplified, and we have used much the same testing procedures as before in the study of various types of amblyopia. Some preliminary results are described in this paper. In assessing their significance and their relationship to single-cell neurophysiological experiments, the limitations of the techniques must be borne in mind. The voltages recorded are obtained from relatively large areas of cortex and there is in general no assurance that in abnormal persons the responding cortical area is identical to that in the normal subject: alterations in the test parameters may also change the cortical area responding. One important illustration of this is the fact that changing the retinal location of the stimulus may change the polarity and the waveform of the recorded response (Halliday and Michael, 1970; Jeffreys, 1971). Since, in our technique, we measure both response amplitude and stimulus-response time, this fact may strongly affect the results. Another important variable is that the stimulus, being designed to maximize the response, will therefore stimulate various classes of cortical cells, each of which has special and different trigger features. A reduction in the amplitude of the response can be due to a selective depression of one class of cell or to a general reduction in activity. Finally, we are recording slow potentials and, although these probably represent the summed inhibitory

post-synaptic potentials (IPSPs) and excitatory post-synaptic potentials (EPSPs) of cortical cells, their relation to signal transmission in the cortex (spike firing) is certainly not simple.

However, with all these caveats, it appears that certain abnormalities present in the VER of amblyopes are explicable in terms of the animal experimentation: that simple extensions of our findings lead to intriguing speculations about the nature of the defects in particular forms of amblyopia: that the test results clearly demonstrate the presence of such properties as suppression and binocularity which may be of interest in treatment: and that the changes in the visual system which occur as a result of treatment also have electrical analogues.

Techniques

VERs were recorded using techniques based on those previously described (Behrman and others, 1972; Arden, 1973). Patients viewed a back-illuminated screen made up of nine hundred 5-mm. squares of "polaroid". Alternate squares had their axes of polarization perpendicular to each other. The viewing eyepiece contained a rotating polaroid disc, so that for each 90° rotation the black and white pattern of the screen appeared to reverse, while the mean luminance remained constant. The angle subtended by each square was varied by moving the screen away from or towards the patient and subtenses of from 9' to 1° could be employed. The screen could also be masked off with various diaphragms, so that square size could be altered independently of screen area. A fixation point (an "LED"—light-emitting diode) was provided at the screen centre, but maintenance of accurate fixation does not affect the results, provided the screen is kept in focus. $\frac{1}{4}$ -wave plates were also inserted in the eyepieces and, by rotating these to fixed angles, the contrast of the pattern could be altered from about 7 to about 99 per cent. The contrast usually employed was 99 per cent. The speed of rotation of the wheel was 1.5–4 Hz., giving from six to sixteen pattern reversals per second. Several modifications were made to the apparatus in testing the amblyopes:

- (a) In some cases the chequerboard screen was replaced by strips of polaroid 5 mm. wide so that the patients saw a pattern of reversing stripes the inclination of which could be varied.
- (b) The patients were tested either with one eye at a time or with both eyes viewing the same target. Thus the responses to either eye could be compared with the response when both eyes were viewing the same target.
- (c) A new eyepiece was constructed in which there were two polaroid discs, one for each eye, which were geared together and rotated at slightly differing speeds. When the patients viewed the screen through this system no binocular fusion of the pattern was possible, since the patterns reversed at differing rates. Photocells monitored the rotations of each wheel and the averager could thus be triggered to record the response from either the right or the left eye, while both sent their independent signals to the cortex. In this way it was possible to test ocular dominance.

Recording

VERs were registered with silver scalp electrodes (standard EEG electrodes) placed 2.5 cm. above theinion and on the vertex. The ear lobe was earthed. The responses were amplified, averaged, and recorded on a physiograph (Medelec MS6). Usually 100 responses were averaged: if responses were small or the noise level high (which is often the case with children), 200 or more responses were averaged and automatically scaled. All responses were repeated to detect variations due to incomplete removal of noise.

Patients

These were selected from the outpatient department of Moorfields Eye Hospital and almost all had been previously examined by the Orthoptic Department. The clinical status was assessed by refraction and the visual acuity was determined with a test-type. Synoptophore measurements of angles of

deviation and binocular state were made. Most of the children were examined during the course of orthoptic treatment; many were examined on several occasions.

Results

DIFFERENTIATION OF CONDITIONS APT TO BE CONFUSED WITH AMBLYOPIA

Chief amongst these conditions is ocular hysteria in which the pattern VER is never abnormal: many such cases are sent to the clinic (not usually from the Orthoptic Department!). Examples of such responses have been shown in previous papers (Berhman and others, 1972; Arden, 1973). The amplitudes of the responses of the two eyes differ by no more than 10 per cent., which is the normal variation, and the timing of the peaks and troughs of the responses are identical for the two eyes. The amplitude of the response is greatly reduced if the visual acuity is reduced by spectacle lenses of 1 dioptre.

In other cases in which amblyopia is suspected the VER indicates the presence of organic lesions. Fig. 1 shows such a case.

FIG. 1 VERs in a child aged 8 suspected of having amblyopia. Probable diagnosis, organic lesion of the central pathways. Visual acuity, right eye 6/36, left eye 6/6. The child's vision was not improved by occlusion. Note that the VERs in the right eye are responses to luminance, not to pattern-reversal

The responses of the right eye are considerably depressed, as much to viewing the screen at 150 cm. (when the cortical response is almost entirely produced by the foveal projection) as to viewing it at 30 cm. (when the cortical response is produced almost entirely by peripheral retina). Note that what response there is, is an oscillation at half the frequency of that produced by stimulating the left eye. This is a response to change in luminance of the squares (which occurs at half the rate of pattern-reversal). Such luminance responses can be detected with our stimulus, because the contrast is very high, but they are usually small and submerged by the second-harmonic pattern-reversal response. The absence of patterned responses, and the fact that the response is absent when the peripheral retinal is stimulated as much as when the fovea is tested, is quite unlike our usual findings with amblyopes. This child's vision remained unaltered by occlusion and he suffered from giant pigmented hairy naevi on the trunk and legs, so that the diagnosis of organic lesion, not amblyopia, can be supported from evidence other than the VER.

AMBLYOPES WITH A HISTORY OF INFANTILE DEFECTS IN IMAGERY

In animal experiments visual defects can be produced by translucent contact lenses fitted during a "critical period". Since analogous conditions exist in man and produce visual defects, it seemed a good preliminary to examine such patients before proceeding to study amblyopes of various kinds in whom there might be additional complications.

Fig. 2 (overleaf) shows the results from a woman who had had a congenital cataract removed, but who was amblyopic. ERGs and other tests of retinal function disclosed no cause for the loss of visual acuity. The pattern VER is abnormal. Not only is the amplitude of the response in the amblyopic eye reduced, but also the waveform is abnormal. The most obvious difference is that the waves seem to be "upside

FIG. 2 VERs in a 27-year-old woman who had had a congenital cataract removed as a child. Visual acuity, right eye 6/6, left eye 6/18. For further details, see text

down" by comparison to the other eye. Since the stimulus is repetitive and sinusoidal and so is the response, the abnormality can be termed a phase change of 180° . These are the two abnormalities of amplitude and phase which we frequently find in amblyopes.

However, the most obvious case in which amblyopia is due to uncorrected optical aberrations is that seen in meridional amblyopia (Mitchell, Freeman, Millodot, and Haegerstrom, 1973), for in such a condition visual acuity is lost only for gratings in one particular orientation associated with the optical error: there can be no question that primary central or motor abnormalities contribute to the defect. Fig. 3 shows such a case. The stimuli are indicated in diagrammatic form. The mark:space ratio of the square wave grating was 1:1.

FIG. 3 VERs in 21-year-old astigmatic subject, who first wore glasses when aged 14. The stimulus patterns were gratings, either vertical, horizontal, or inclined between 10 and 15° to the horizontal, as indicated. Correction was -2.75 D sph., $+0.5$ D. cyl., axis 180° . Note that in the normal (vertical) meridian defocusing by 0.5 D (centre column) reduces response amplitude, and the "amblyopic" horizontal meridian is the one with the smallest refractive error (Fiorentini and Maffei, 1973)

The patient was a 21-year-old girl who had uncorrected myopia and astigmatism and had not worn glasses till she was 14 years old. She was quite unaware of any visual defect. The left-hand column shows responses to the patterns in various orientations: in the lower two traces the stripes inclined $\pm(10-15^\circ)$ from the horizontal. It is quite clear that, for horizontal gratings, the responses are considerably smaller than for vertical gratings. In normal subjects the amplitudes are the same (Maffei and Campbell, 1970). Even slight tilts of the grating cause increased amplitudes of the VER.

Note that the timing of the responses, *i.e.* the relationship of the trigger on the averager to the black-white transitions, depends up on the orientation of the pattern: thus the peaks and troughs do not have the same delays in all the traces and it is probable that the horizontal stripes give a response which is delayed in respect of the vertical stripes.

Fig. 3 also shows the effect on the VER of altering refraction. For the vertical stripes, a change in refraction of 0.5 D causes the response amplitude to drop considerably to approach that produced by the horizontal stripes. Altering the refraction in the case of the horizontal stripes scarcely affects the response. In this subject, the correction was -2.75 D sph., with $+0.5$ D cyl., axis 180° . It is noteworthy that meridional amblyopia can be demonstrated with such a small additional correction: in the cases already reported, with results similar to those shown in Fig. 2 (Fiorentini and Maffei, 1973), the astigmatism was much greater. Of course, we have no knowledge of the correction our subject required during her early years when the defect was acquired.

RESULTS IN A SERIES OF AMBLYOPES

Unocular testing

We have investigated responses in a series of 42 amblyopes, mostly children: thirty were children aged 5 to 11 years; the rest were adults aged 16 to 50 years. Of these patients two were diagnosed (after scrutinizing the VERs) as having organic disease and were excluded. In three the records were so poor as to be ambiguous. Some of the results in the remaining 37 patients are presented in the Table.

Table *Summary of results in amblyopes*

Two cases were excluded (because of organic disease) and in three further cases the records were too poor for analysis

Classification	No. of patients	Amplitude				Phase			
		Reduced		Normal		Abnormal		Normal	
		Visual acuity		Visual acuity					
		Worse than 6/18	Better than 6/18	Worse than 6/18	Better than 6/18	B.V. absent	B.V. present	B.V. present	B.V. absent
Anisometropia	14	11	1	3	0	3	18	2*	0
With deviation	6	2	1*	3	1	6	0	0	0
Squint									
With equal or no refractive error	7	2	1**	3	1*	4	2	1**	0
With anomalous retinal correspondence	6	3	0	3	0	0	6	0	0
With eccentric fixation	4	3	0	1	0	4	0	0	0
Totals (excluding with* or **) patients	37	21	1	13	1	17	26	0	0

** Case of alternating sursumduction with family history of congenital abnormalities. No apparent ocular deviation, or abnormality of binocular vision when originally seen in Orthoptic Department at 3 yrs old.

* Recent total occlusion may have caused reduction in VER amplitude in good eye and disturbed binocular function

Note that in 21 out of 22 cases, reduced acuity is associated with VER reduced by comparison with the fellow eye, while in 13 out of 14 cases with acuity 6/18, there is no such reduction defined as a 40 per cent. decrease compared with the non-amblyopic eye.

VER phases are normal (similar in each eye) when binocular vision is present (26 observations), and abnormal in 12 out of 20 cases in which binocular vision was absent. However, in the 3 cases in which phase was abnormal but binocular vision was said to be present, there are complicating factors, see asterisks.

Five types of amblyopia have been distinguished, but this does not mean that in each type the patients suffered from identical conditions. For example, in the group with equal

or no refractive error and squint, one patient had alternating sursumduction. Many of the patients were seen on several occasions. In some this was necessary because the first records obtained were too poor for analysis and the first test had to be considered as a training session. However, we attempted to follow the progress of the patients as they were treated. This met with limited success, and we discovered that, after relatively prolonged examination (we were at the time attempting to discover what stimulus parameters were of interest), a significant proportion of children suffered (according to their mothers), from more disturbance—*e.g.* crying, complaining of headaches, drowsiness—than would be expected of children simply attending an orthoptic clinic. VERs were recorded as the patient viewed the screen monocularly, the other part of the binocular eyepiece being blanked out or the second eye occluded with a tight patch.

The VER amplitudes measured varied greatly, more so in these children than in the adults previously tested (Behrman and others, 1972), and measurements of voltage are therefore not useful. However, the relative amplitude of response in the two eyes is a much more reliable index, as previously shown, and should not exceed 10 per cent. In these children, where responses were noisier than in adults, we have arbitrarily considered that a response was depressed, relative to the other eye, if it was depressed by 40 per cent. or more, and it is on this basis that the figures in columns 2 to 5 of the Table were obtained. In some cases it was only when small subtense squares were used (foveal responses) that the response was depressed. The worse the visual acuity the larger the square size which gives an abnormal response tends to be. An additional complicating factor is that prolonged occlusion of the good eye for treatment can reduce its response, and most of these children had been treated by occlusion (see below). However, the results are fairly clear-cut and show that there is a marked depression of the amplitude of the VER only if the visual acuity is worse than 6/18. This is to be expected from our previous results with organic disease.

The other variable which could be measured on the traces was the relative phase of the amblyopic and fellow eye. Here the results are extremely suggestive, for in the overwhelming majority of the patients without binocular vision, the phase of the responses differed from the normal, while all those with normal phases had binocular vision. Binocular vision was considered to be present if there was fusion, either on the synoptophore, even if only over a small range or with the "Wirt Fly test": so the "binocular vision present" group includes those without central fusion. It was observed that all the patients with anomalous retinal correspondence had normal phases, while none of the eccentric fixation group or anisometropes with deviation had normal phases or binocular vision. There are three exceptions (col. 8), but of these one was the case of alternating sursumduction and the other two were anisometropes without deviation who had recently been occluded. They were said to have binocular vision, but were not tested for fusion, etc., on the day of the VER, and we have evidence (see below) that occlusion does affect the VER as well as vision!

If these three cases are ignored, our results point to a simple rule: if binocular vision is present, the VER from each eye has the same timing; if binocular vision is not present, the timing of the two eyes is different.

This series of tests provided other information not included in the Table. In eight cases, all anisometropes, we were able to follow changes in the VER associated with treatment. Thus we noted increases (and decreases) of the VER associated with changes in visual acuity in the amblyopic eye and alterations in the VER of patched eyes and changes in phase associated with establishment or loss of binocularity.

One such case is illustrated by the records shown in Fig. 4 (opposite).

FIG. 4 VERs in a 5-year-old anisometropic amblyope. The inserts in the Figure gives the dates of the examination, visual acuity, screen-patient distance, and occlusion history. The square symbols indicate the area of the patterned screen viewed by the patient. The outer square represents the full extent of the screen, and the black infilling, the part of the screen which was masked from the patient's view. For all tests other than those indicated, the patient regarded the entire screen. For further details see text

The child, a 5-year-old anisometropes, was seen before any treatment was given, when the VER in the affected eye was nearly absent. After 3 months' occlusion, visual acuity in the amblyopic eye was improved and the VER could be recorded from this eye: no response was now seen from the occluded eye, although vision in the occluded eye was still 6/9. Note that the VER from the amblyopic eye is phase-reversed. After 10 months' patching with a translucent occluder, there was no further improvement in vision and the child no longer showed fusion. VERs were recordable from both eyes, but the amblyopic eye was phase-reversed: the amplitudes of responses at both 70 and 150 cm. were the same (the effect of binocular stimulation and occluding part of the field are considered later). At this stage the child had no binocular vision and it was considered that further patching would serve no purpose. The treatment was discontinued and the visual acuity in the amblyopic eye fell to 6/24, but binocularity was re-established.

The VERs are now in phase, and the amplitude at 70 cm. is the same for each, but at 150 cm. the responses from the left eye are obviously reduced. The variations in waveform and in amplitude and the noise on the records are rather better than the average obtained in young children, but are not exceptional.

Other points have been investigated in a few patients. With crude electrode placements on the midline, frominion to vertex, we were unable to detect any difference in the spatial distribution of the response from normal and amblyopic eyes which gave responses which are "out of phase". In one case we tested the effect of stimulating the upper and lower

halves of the visual fields separately, since it is known (Halliday and Michael, 1970; Jeffereys, 1971) that the phase of the response may alter when this is done, probably because a different cortical region is activated. In our case the reversal of phase occurred for the normal eye but not for the amblyopic eye.

BINOCULAR STIMULATION

This was carried out in some of the patients. Two techniques were available, as described under "Methods": in one, the two eyes viewed the same target, and in the other, since two polaroid discs rotating at different speeds were used, the responses to the two eyes should be independent. The reason for this test was that many cells in the visual cortex are stimulated by either eye. In a population of cells equally affected by either eye, the response to both (if saturating stimuli are employed) should be either the same as to one eye or slightly larger. Campbell and others (1966) give the enlargement as 1.4. On the other hand, if the cortical cells are effectively uniocular, then the responses from either eye should add algebraically. Many other variations are, of course, possible. It might be that, in amblyopia, the response to one eye is suppressed: then the response to binocular stimulation would be that of the dominant eye.

We have seen a number of variations which may be interpreted in this way. For example, in Fig. 4, during the third examination, a binocular test was performed on the patient: the amblyopic eye apparently generated the binocular response (the other normal eye had been patched for many months). It seems from this result that the amblyopic eye can suppress responses from the normal eye in this child. Certainly, therefore, some binocular interaction was possible and, later, in this case, binocular vision was re-established. The reverse of this finding has been observed where the response to a binocularly-viewed target is absent: it may then be that the out-of-phase responses from the two eyes are summing algebraically at the electrodes. In such a case there may be no interaction between the two eyes at all.

The problem can also be investigated with the second type of binocular target, in which fusion is impossible. In this the response to each stimulus alone should be reduced when both are presented together, since some cells, previously affected by the single target (presented say to the right eye), will be responding to the stimulation of the left eye, when there is two-eyed viewing of the non-fusible targets. This is what happens in normal subjects (see Fig. 5), but in amblyopes a different result is obtained. Fig. 5 shows an anisometrope with good visual acuity and binocular vision. The responses to each eye, tested alone, are equal and large. In the two-eye test, the response to the right eye is scarcely changed, whereas that to the amblyopic left eye is markedly reduced: responses from the left eye are being suppressed by the right eye. This sort of suppression has been observed before in experimental situations (Cobb, Morton, and Ettlinger, 1967). However, in amblyopes, it is clearly evident and no special tests are required to demonstrate it. In more severe cases than that shown in Fig. 5, the uniocular response from the amblyopic eye may be much reduced if the normal eye is not closed and light excluded. Another form of suppression encountered in amblyopes, which has been previously reported (Arden, 1973), appears to be a suppression of the central retinal response by the periphery. An example is shown in Fig. 4. On the third visit, we obtained VERs from the child when the stimulus activated only the central 1° or so of the retina surrounding the macula (at 150 cm.), by using stimuli of small-square size, and we also recorded responses to larger areas of retina, with the screen placed nearer to the patient (at 70 cm.) However, when the screen was masked off so that only the central 5° of retina was stimulated, the response in the amblyopic eye failed. It appears

FIG. 5 Responses obtained with each eye viewing pattern-reversing stimuli but with a slightly different frequency of pattern-reversal for each eye. The timing of the responses for the two eyes cannot be compared. For further details see text

that, in the circumstances that both macula and paramacula are stimulated, the response is suppressed, although the peripheral retina and the macula alone can each produce responses.

The use of binocular stimulation was tried in an attempt to examine amblyopes with a view to deciding prognosis and treatment, but, as described above, we have found many variants of a basic pattern. One further finding, which we have seen on several occasions, is a binocular response which is many times greater than the response to one eye individually: there seems no simple interpretation of this observation and we are continuing to analyse binocular responses. We have, however, without such tests, established that there may be a simple evoked potential correlate of binocularity.

Conclusions

Although it is likely that human amblyopia is produced by the same mechanisms as those that cause visual defects in the eyes of kittens deprived of accurate form vision, proof is lacking. Thus it is of some interest that, in cases in which the defect is demonstrably the same as in animal experiments (Figs 2 and 3), VER abnormalities are found in human subjects and are of the same nature as in the other common forms of amblyopia.

In their original studies on deprivation, Wiesel and Hubel (1963, 1965b) found that there appeared to be a loss of the cortical cells which normally respond to the eye that had been deprived of form vision. However, more recent work suggests that instead (or in addition) cells which normally would have responded to one class of visual input are functionally changed, so that their trigger features are altered. Thus, kittens reared in an environment of vertical stripes have cortical cells which respond only to vertical stripes (Hirsch and

Spinelli, 1971; Blakemore, 1974; Blakemore and Cooper, 1970). It seems possible that isometropic amblyopia arises simply as a result of an analogous mechanism (as discussed by Ikeda, 1974). The VER findings are very suggestive, for it appears that, when the fovea is stimulated concurrently with the parafovea, there is little cortical response, though either area stimulated alone can produce responses, and therefore the parafoveal projection can in some way influence the foveal projection.*

The VER has been used to investigate amblyopia before, but usually utilizing only flashes as a stimulus. As Spekreijse and others (1972) pointed out, such stimulus conditions do not disclose VER abnormalities, nor would one expect them to do so (although claims to the contrary are still made, *e.g.* Tsutsui, Nakamura, Takenaka, and Fukai, 1973). The only study using constant mean luminance, pattern-evoked potentials is that of Spekreijse and others (1972). In this the evoked potential was used as an index of psychophysical function and it was shown that in one patient the amplitude of the responses was reduced, the waveform changed, the contrast sensitivity reduced, and the response originated less from the foveal than the parafoveal region. Suppression and binocular interaction were demonstrated. However, the investigation was limited to one adult anisometrope with stereopsis. The results of this detailed investigation are consistent with our much cruder studies, and study of the waveform of the responses suggests that, though their technique was rather different from ours, Spekreijse and others (1972) also saw a phenomenon which would account for what we describe as a "phase-reversal".

In clinical work, cruder techniques must be employed, and our method does not permit us to be sure whether the changes in amplitude and timing of the VERs are caused by changes in the number of cortical cells responding, or changes in their location in the brain, or in their manner of functioning. Our preliminary results provide clear-cut distinctions between different types of amblyopia and, in particular, whether or not there is binocular vision. A simple superficial explanation of our result is easy to give: when binocular vision occurs, some cells in the brain must handle data from each eye in the same way, at the same time, and at the same location. If the VERs produced by identical stimulation of each eye are out of phase, it is unlikely, from any theoretical point of view, that these conditions are met.

Assessment of binocularity by an objective test would have some clinical application and there is some evidence in our results that a phase-reversed VER before treatment may have some prognostic significance. We are continuing our investigations with this in mind and are also attempting to quantify our measurement of binocular interaction, using the VER.

* NOTE ADDED IN PROOF

Recently Lawwill, Meur, and Howard (1973) have obtained psychophysical evidence for lateral cortical suppression in amblyopes, which is analogous to our findings.

Discussion

MEIN In view of the fact that the visually evoked responses can change in treatment or non-treatment, are they any use in making a prognosis in cases of amblyopia?

ARDEN This very much depends on the individual case. If I were expecting to patch the child for one whole year it would be a good idea to be sure that this was a true case of amblyopia and not one due to an organic cause. Certainly, if there is any question of hysteria, it is much better not to treat the child at all but rather to reassure the parents and send the child away.