Pattern evoked potentials in awake rhesus monkeys

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The pattern evoked potential (EP) in man to checkerboard stimulation has been shown to consist of various components originating in different regions of the visual cortex. Surface recordings, however, cannot unambiguously localize the sources of these components; for precision, depth recordings seem to be indicated. Considering the close correspondence in cortical architecture to man, rhesus monkeys (Macaca mulatta) seem to be a suitable animal for such experiments. However, Padmos et al. demonstrated that in monkeys anesthetized by pentobarbital (Nembutal), no pattern EP as found in man could be recorded. The present experiments were carried out to investigate whether the lack of contrast-specific EPs in monkey can be attributed to the effects of anesthesia. Four rhesus monkeys were trained to fixate at a television screen on which checkerboard and bar patterns of various sizes could be presented. The results from this study demonstrate that in monkey as in man, pattern EPs can be obtained that can be distinguished from those evoked by luminance variations. Therefore the awake rhesus monkey seems to be a suitable experimental model in the search for the origin of the pattern EP.

Key words: pattern evoked potential, anesthetics, rhesus monkeys, visual cortex, surface recording, behaving

In man, checkerboard patterns elicit a visually evoked cortical potential essentially different from that in response to stimulation with homogeneous illuminated fields. The difference can be deduced from the fact that the appearance and disappearance of a pattern generate differently shaped evoked potentials (EPs), although from a luminance point of view the onset of the pattern cannot be distinguished from the pattern offset. Thus, if luminance is the prime parameter, identical responses would be expected to both pattern onset and offset. Of the two response types, the pattern onset response has been studied more extensively. In the positive-negative-positive (PNP) complex of this response, three components can be distinguished on the basis of topological representation. The first positive component (denoted CI) is generally accepted to have a striate origin, whereas the other two components (the negative CII and the positive CIII) are assumed to be of extrastriate origin.

One of the reasons for the growing interest in the pattern EP is its usefulness in clinical practice. For example, in the pattern EP mediated through an amblyopic eye, only one component (CI) of the PNP complex is typically present in contrast to the normal PNP complex generated after stimulation of the fellow eye. Pattern EPs are also applied in the diagnosis of certain demyelinating diseases such as multiple sclerosis (MS). The pattern onset responses in MS patients fre-
Fig. 1. EP recording of a behaving rhesus monkey in primate chair. The monkey fixates a red LED situated in the middle of a television screen on which a checkerboard pattern (bottom left) is presented for 400 msec, followed by a homogeneous field for 600 msec (bottom right) without a change in mean luminance level.

Thus far the neuronal bases of various components in the pattern EP have not been established. Such a search has to be carried out with invasive recording techniques. The rhesus monkey (Macaca mulatta) seems to be a suitable animal model for these experiments because of its highly developed visual system and the resemblance to the human visual cortex. In a previous study an attempt was made to determine with conventional scalp recordings whether the rhesus monkey has pattern and luminance responses as found in man. The study was undertaken in animals anesthetized with pentobarbital (Nembutal). Under these conditions, no genuine contrast EPs could be detected, since these responses could not be distinguished from those evoked by pure luminance stimulation. From these findings it was concluded that pattern EPs with a contrast origin could not be recorded in anesthetized monkeys. There is an indication that anesthesia may also affect the luminance response. In a study by Hughes, for example, a 10 mg/kg injection of pentobarbital sub-
Fig. 2. Training paradigm. After a warning tone, the monkey is trained to fixate a red LED of 6 min arc in the center of the screen. After brightness increment of this fixation light, the monkey presses the response key to receive an apple juice reward. Stimulus presentation is independent of the training task. The EPs are recorded from the instant the monkey fixates the screen. The averager is interrupted when the monkey gazes away.

Moreover, substantially delayed the luminance EP and also altered the polarity of the response components. More recent reports therefore have been concerned with EPs in awake monkeys.10–13 The contrast results from these studies, however, are ambiguous, since the pattern stimuli used were presented under stimulus flash conditions. Spekreijse et al.14 demonstrated that in man a flashed-on pattern does not generate a contrast EP but rather an enhanced luminance EP. Thus the presence of an EP to a patterned flash may not be conclusive about the luminance or contrast origin of the response. We therefore decided in our search for pattern EPs in monkey to use (1) awake animals that were trained to fixate and accommodate at the screen on which the patterns were presented and (2) pattern presentations without overall changes in mean luminance level. A similar approach has been chosen by Coppola and Nakamura.15

Methods

Stimulus. Both the pattern and luminance stimuli were generated on a television screen subtending 9 by 12 degrees of visual angle with a mean luminance of 63 cd/m². In the middle of the screen was positioned a red light-emitting diode (LED) subtending 6 min arc, which the monkey was required to fixate. The monkeys were seated at a distance of 1.7 m from the screen. Pattern stimulation consisted of abruptly appearing and disappearing checkerboard and bar patterns at a constant mean luminance level. Luminance stimulation consisted of a stepwise increase and decrease of the whole field luminance, having the same time profile as the pattern onset and offset stimuli. The pattern onset-offset rate was low to avoid contamination of the appearance responses by the disappearance responses. Simultaneous
monkey man

Fig. 3. Checkerboard and bar pattern EPs derived from monkey and man with surface electrodes attached on the occiput as indicated. Top half of the figure gives the responses to small (12 min arc) and large (48 min arc) checks. Bottom half displays the responses to narrow (4 min arc) and wide (48 min arc) horizontal bars. The contrast in all patterns is close to 100%, field size 9 by 12 degrees. 

Appearance of the pattern. Onset of pattern is achieved by increase in the luminance of one set of checks and simultaneous decrease in that of the other set to the same extent. In this way the mean luminance of the stimulus field remains constant during each stimulus cycle.

Disappearance of the pattern. Luminance of the two sets of checks return to the same value of $L_o = 63 \text{ cd/m}^2$. Patterns were presented for 400 msec once per 1000 msec.

Animal training. Four mature rhesus monkeys, three female and one male (3 to 5.5 kg), with normal visual acuity were used. During weekdays, the monkeys were deprived of liquid and were trained two times a day. At the beginning of a training session, the monkey was placed in a primate chair (Fig. 1). The training starts with the presentation of a 350 Hz tone for 0.5 sec. This tone is followed after 1 to 8 sec at random by a 2 sec brightness increase of the LED. (While the monkeys were inexperienced, a light spot subtending 1 degree of visual angle was used.) As long as the LED is on, the monkey can depress a handle bar and receive 0.25 cc of apple juice delivered through a tube located in the proximity of its mouth. Later the monkey is allowed to press the handle bar only once per LED increment. After a random period of 5 to 10 sec this sequence is repeated (Fig. 2). If, however, the response key is depressed when the LED is not on, it takes longer for the next sequence to start again. The monkeys were regarded as suitably trained for the experimental sessions when an 85% correct response level was reached.

Anesthetized preparation. One of the monkeys was premedicated with sernylan (2 mg/kg i.m. in a 20 mg/ml solution) and atropine (1 ml i.m. in a 0.5 mg/ml solution). Pentobarbital anesthesia was initiated by an injection of 30 mg/kg i.v. followed by an infusion of 4 mg/kg/hr during initial stages of the preparation. The monkey was placed in a stereotaxic apparatus after the external auditory chan-
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Fig. 4. Peak-to-peak amplitude of the pattern onset EP is plotted as a function of the size of the spatial elements in the structured field. In the awake state (situation C), the monkey gave the largest response for square-wave gratings with a bar width of about 4 min arc. For checkerboard stimulation, a double-peaked amplitude vs. check-size curve was typically obtained with peak responses occurring at about 4 and 12 min arc. Under pentobarbital anesthesia (situations A and B; see Fig. 6), the pattern EPs to fine elements were severely reduced; responses to coarse structures were unaltered. After reduction of the depth of anesthesia, preference for element size was gradually regained.

Results and discussion

Fig. 3 shows for comparison the EPs derived in monkey (left) and man (right) after the appearance and disappearance of either checkerboard (upper traces) or horizontal bar (lower traces) patterns. We believe that these responses are conclusively of contrast origin. As indicated in the traces at the bottom of Fig. 3, the onset and offset of the pattern was not accompanied by an overall change in luminance. If local luminance changes would
Fig. 5. Pattern (top traces) and luminance (middle traces) EPs from anesthetized monkeys. The left-hand EPs are from the same monkey as in Figs. 3 and 4; the right-hand responses are from a previous study.8 The pattern responses (upper traces) were obtained by stimulation with checkerboard patterns (solid lines) or horizontal bar patterns (dashed lines, left side). Luminance EPs were generated by a 50% square-wave modulated homogeneous field of the same size, time profile, and mean luminance as the corresponding pattern stimuli. In the bottom trace, pattern (solid lines) and luminance (dotted lines) EPs are plotted together. On and off refer to pattern onset and offset or to stepwise increase and decrease of the luminance stimulus. Left-hand EPs were derived with a surface electrode 5 mm above the anatomical inion. Right-hand EPs were derived with an electrode over the foveal projection (position T6; see also Fig. 8). Mean luminance at the left amounts to 63 cd/m², at the right to 20 cd/m².

have generated the EPs depicted, the same response would have been expected at the appearance as well as at the disappearance of the pattern. The EPs to the onset of the patterns clearly differed, however, in shape from those to the offset of the patterns. Furthermore, larger responses were generated by the presentation of small checks (12 min arc, top records) than by the onset of large checks (48 min arc, bottom curves). If these EPs were generated by local luminance variations, a greater response amplitude might be expected for larger check sizes, which is in line with a simple receptive field model without an antagonistic center/surround organization. On the basis of these arguments, we conclude that in awake monkeys, as in man, EPs to pattern presentation can be derived that have a contrast origin. There are, however, differences in the dependence on stimulus parameters. For example, narrow bars generate EPs in monkey with about the same amplitude as checkerboards. In man, however, bar patterns are generally a much less effective stimulus.

In Fig. 4, the amplitude of the positive component in the pattern onset response is plotted as a function of check size (curve C, solid lines) and bar width (curve C, dashed lines). The checkerboard onset response had two maxima at checks of 4 and 12 min arc, respectively. In man, typically only one optimal check size is found at about 10 to 15 min arc. The bar pattern response in monkey had only one pronounced maximum value, occurring at a much lower bar width (4 min arc) than that in man. One may bear in mind that single-unit studies in the striate cortex of awake monkeys have revealed neurons responding optimally to square-wave gratings with bar widths of 3 to 7.5 min arc.16 Fig. 5 shows the EPs recorded in monkey under
Fig. 6. Power spectra of the monkey EEG (ionion-vertex derivation) as a function of depth of pentobarbital anesthesia. All spectra are normalized. The power in the lowest spectrum is one-tenth of that in the topmost spectrum. During normal anesthesia (situation A), the background EEG is severely reduced; the more so, the higher the frequency. From the moment of disconnecting the pentobarbital infusion (spectrum indicated by a star), there was a gradual increase of especially the middle and higher frequency components in the EEG (situation B). After about 1 hr the monkey awakened, yielding the topmost power spectra (situation C).

The absence of a contrast-specific EP under pentobarbital anesthesia was also evident when the peak-to-peak amplitude of the pattern onset response was plotted as a function of check size or bar width (Fig. 4). At normal depths of narcosis (situation A), the previously observed pattern specificity was absent, and the largest responses were obtained for the coarsest elements. Fine elements generated much smaller responses than those in the awake situation, whereas large elements generated about the same amplitude response in both situations. When more superficial depths of anesthesia were applied (situation B), a preference for smaller check sizes was regained although it was not as pronounced as in the fully awake state (situation C).

Since the response is altered so dramatically under anesthesia and since it seems unlikely that this reflects mainly variation in peripheral processing, it was also determined whether this overall change in response would be paralleled in the EEG signal itself. The power spectra of the background EEG are depicted in Fig. 6. At the deepest level of anesthesia (situation A), the high-frequency
content was the most severely reduced; even at the superficial level of anesthesia (situation B), the background EEG had not regained the high-frequency content present in the fully awake state (situation C).

Our finding that in monkey under pentobarbital anesthesia no genuine contrast EPs can be recorded may be related to the recent report that in children under enflurane (Ethrane) anesthesia the EP amplitude to fine gratings is more attenuated than for coarse ones.17

In reaching a final conclusion, one control seems indicated: if pattern onset and pattern offset are accompanied by overall changes in luminance, the asymmetric response obtained in our study could be attributed to this artifact. To exclude this possibility, a translucent screen was placed in front of the television screen in such a way that the checkerboard pattern could still be observed but that the edges of the checks were faded substantially. As can be seen in Fig. 7 (top trace), this situation did not generate any response at all. This proves that our stimulus was not contaminated by overall changes in luminance, since such changes elicit a well-defined response (Fig. 7). This result also indicates that in awake monkeys, as in man,18 the pattern EPs are primarily generated by the contrast immediately across the edges. The pattern EP depicted at the bottom of Fig. 7 was obtained without the edges faded.

As a final control, we tested fixation by presenting two patterns simultaneously, one in the left half of the visual field and one in the right half. The stimulus presentation times (top traces, Fig. 8) were selected so that the instants of onset and offset of the two patterns had no fixed time relation to each other under these conditions. From each of the electrodes two responses could be derived simultaneously, yielding the scalp distribution of the contrast EPs, as seen in Fig. 8. Since this distribution has a clear contralateral representation, it can be concluded that our monkeys fixated well during the recording session. Also, control experiments with a pattern reversal stimulus showed a clear contralateral representation. This finding is not in conflict with the pseudoipsilateral origin of the reversal EPs as described by Halliday in human beings (for a conclusive review see the edited discussion in Barber,19 p. 292 ff.), since in rhesus monkey the foveal representation is displaced toward the temporal lobes.

Conclusion
1. Awake rhesus monkey, like man, generates genuine contrast responses to pattern stimulation.
2. The response to pattern appearance can be distinguished clearly from that to pattern disappearance.
Fig. 8. Fixation control. Responses to simultaneous left-half and right-half field stimulation. Both the right-half (solid) and left-half (dashed) visual fields were simultaneously stimulated with an appearing and disappearing checkerboard (check size 12 min arc). Presentation times of the two patterns were chosen in such a way that the onset-offset instants of the two patterns had no fixed time relation (top). In this way, the responses from each of the electrodes to simultaneous right-half and left-half field stimulation could be averaged separately. The right temporal electrode (T6) yielded greater amplitude responses from left-half field stimulation (dotted line); the left temporal electrode (T5) yielded mainly from right-half field (solid line) stimulation. At the midline electrode (inion), the responses to both half fields were approximately equal.

3. The pattern EP amplitude varies with the size of the elements in the pattern.

4. The use of anesthesia abolishes the presence of contrast-specific components in the pattern EP.

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