

# Pattern-onset stimulation boosts central multifocal VEP responses

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Multifocal visual evoked potentials (VEP) allow one to assess whether stimulation at specific visual field locations elicits cortical activity; it might therefore enable us to conduct objective visual field perimetry. However, due to the cortical folding, which differs markedly between subjects, a particular electroencephalogram generator may fail to project signal on some recording electrodes. This may lead to false alarms for potential scotomata. Here we compare pattern-reversal and pattern-onset stimulation in their efficacy to activate the visual cortex and recorded mfVEPs to 60 locations comprising a visual field of 44° diameter. We report three main findings: (1) Pattern-onset compared to pattern-reversal enhances the amplitude by 30% for stimulation of the central visual field (<10° radius), while evoking 30% less response in the periphery (>15°). (2) Although pattern-onset and pattern-reversal responses differ markedly in their eccentricity dependence, they have a similar topographical distribution. (3) By combining both stimuli, the number of false positives was reduced to less than 1.5% of the visual field locations tested. We conclude that pattern-onset and pattern-reversal activate identical visual cortical areas but target different neural mechanisms within these areas. Furthermore, pattern-onset stimulation greatly increases the sensitivity of the mfVEP to assess the cortical representation of the central 10° of the visual field.

**Keywords:** human, visual cortex, topography, visual evoked potentials, multifocal, onset, reversal

## Introduction

Multifocal visual evoked potentials (mfVEPs) enable us to record, within a short time interval, cortical responses from a great number of distinct visual field locations. Therefore, they provide a powerful tool to assess objectively visual performance at focal visual field locations (Sutter, 1991; Baseler, Sutter, Klein, & Carney, 1994; Klistorner, Graham, Grigg, & Billson, 1998; Hood & Zhang, 2000); a combination of mfERGs and mfVEPs might even allow us to uncover the origin of a visual field defect and to assess the nature of visual pathway abnormalities. The practicability of this promising technique, however, is greatly reduced by the cortical convolutions and their variability among subjects (Hood & Zhang, 2000), which was already demonstrated in the first mfVEP study (Baseler et al., 1994). As a consequence of this convolution, activity at some cortical locations will project onto a derivation, while activity at others will fail to project onto this particular derivation. These latter locations will therefore appear to be silent, and the corresponding visual field locations will spuriously appear as scotomata in the resulting visual field map.

Several approaches have been advanced to cope with the local loss of signal from particular cortical locations.

Some researchers (Graham, Klistorner, Grigg, & Billson, 2000; Hood, Zhang, Greenstein, Kangovi, & Odel, 2000) took advantage of the fact that corresponding locations in the retina of both eyes are represented at similar locations in the visual cortex. They rightly concluded that differences in the visual field maps of the two eyes must be due to veridical scotomata and thus established an objective visual field perimetry based on the interocular comparison of mfVEP responses. Unfortunately, this approach is limited to the detection of only a subset of visual field defects, namely those which originate in the retina or optic nerve and will consequently not result in homonymous scotomata. For the detection of homonymous scotomata, it is vital to fill the apparently silent cortical locations and their corresponding visual field locations. This has been addressed with an increased number of physical derivations and by creating virtual derivations by re-referencing the physical derivations (Klistorner & Graham, 2000; Hood, Zhang, Hong, & Chen, 2002). The approach was successful in reducing the number of spurious scotomata, but still failed to completely eliminate the problem of false positives. To increase the specificity of the detection of visual field defects, a veridical scotoma has therefore been defined as the contiguous expanse of three silent visual field

locations (Goldberg, Graham & Klistorner, 2002). However, this greatly reduces the spatial resolution of the mfVEP-based visual field perimetry.

In the present study, we introduced pattern-onset-offset stimulation to improve the accuracy of mfVEP-based visual field maps. This approach is motivated by results from classical VEPs and is based on the following rationale: (1) From classical VEP studies, brief pattern-onset pulses are known to evoke greater responses than pattern-reversal stimulation and might therefore have the potential to increase the signal amplitude in mfVEP experiments (Howe, Mitchell & Robson, 1981; Parry, Murray & Hadjizenonos, 1999). (2) It is widely accepted that the pattern-reversal mfVEP is generated mainly in V1 (Slotnick, Klein, Carney, Sutter, & Dastamalchi, 1999). In contrast, there are at present no clear views on the origin of the pattern-onset mfVEP. As classical pattern-onset VEPs originate in both striate and extrastriate cortex (reviewed in Di Russo, Martinez, Sereno, Pitzalis, & Hillyard, 2002), the pattern-onset mfVEP might to a considerable extent originate in extrastriate areas. This possibility opens an exciting perspective: Striate and extrastriate areas differ, as a matter of course, in their convolution. Consequently, visual field representations, which do not project onto a particular derivation when they activate V1, might project onto this derivation when they activate extrastriate cortex. Therefore a signal drop-out during pattern-reversal stimulation of V1 might, for example, be filled in with pattern-onset responses from V2.

We compared mfVEP responses to pattern-reversal and to pattern-onset stimulation and report greatly enhanced signals in the central visual field after pattern-onset stimulation. The topographical layout of the response to the two stimuli, however, is remarkably similar, which implies that their generators reside in the same visual area. These two seemingly contradictory findings are taken as evidence that the two stimulus types target different neuronal mechanisms in the same visual area.

## Methods

### Subjects

Six subjects aged 20 to 25 years with normal vision (visual acuity >1.0) gave their written consent to participate in the study. The procedures followed the tenets of the declaration of Helsinki and the protocol was approved by the ethics committee of the University of Freiburg, Germany.

### Stimuli

VERIS 4.8 (Electro-Diagnostic Imaging [EDI], San Mateo, CA) was used for stimulus delivery and electrophysiological recordings. The stimulus display, a

circular dartboard-checkerboard pattern (mean luminance 27 cd/m<sup>2</sup>; contrast 98%), was viewed from a distance of 30 cm and covered 44° of visual angle. Sixty fields of this display were stimulated independently according to an m-sequence with 2<sup>15</sup>-1 elements. Each field comprised a checkerboard consisting of 4 x 4 checks. The radial extent of the fields was scaled with eccentricity from 1.5° in the center to 7° in the periphery. M-sequences consist of a pseudo-random succession of 0 and 1 states. For pattern-reversal stimulation, these two states were represented by two contrast inverted checkerboard fields. For pattern-onset stimulation, state 0 was represented by a succession of two gray fields, while state 1 was represented by a succession of checkerboard pattern and gray. It should be noted that the states last twice as long for pattern-onset stimulation than for pattern-reversal stimulation, as pattern-onset/offset comprises a frame of pattern *plus* a frame of uniform gray for the elemental state. Therefore, a single block of pattern-onset stimulation lasted about 14 min, while a single block of pattern-reversal stimulation lasted about 7 min. Four blocks comprised a recording session, two for each stimulus. The blocks were arranged according to an ABBA design. The blocks were broken up into overlapping segments each lasting about 27 s. All mfVEPs were obtained with binocular stimulation.

## Electrophysiological Recordings

The mfVEPs were recorded with four gold cup electrodes. The electrodes were placed following previous studies (e.g., Hood et al., 2002), 4 cm above the inion (here named Oz), and 4 cm lateral to the location 1 cm above the inion (named OL and OR for the left and right derivation, respectively) and were referenced to an electrode at the inion (named Iz). The EEG was amplified with a physiological amplifier (Toennies) and band-pass filtered (low- and high-frequency cutoffs: 3 and 70 Hz).

## Analysis and Statistics

Re-referencing of the three physical derivations as in previous studies (e.g., Hood et al., 2002) yielded an additional three derivations: physical derivations: (1) OL, (2) Oz, and (3) OR vs. Iz; re-referenced derivations: (4) OL vs. OR, (5) Oz vs. OR, and (6) OR vs. Oz.

First- and second-order kernels for pattern-onset and pattern-reversal stimulation, respectively, were extracted using VERIS 4.8 (EDI). Spatial smoothing and artifact rejection features available in VERIS were not used. All subsequent analysis was performed with IGOR 4.0 (WaveMetrics, Lake Oswego, OR, USA). Traces were digitally filtered (0–30 Hz).

To assess signal presence we evaluated the signal-to-noise ratio (SNR) as described by Zhang, Hood, Chen, and Hong (2002) using their “mean noise-window SNR.” First, the records from the two blocks for each stimulus

were averaged. Then the SNR for each  $i$ -th sector (of the  $n = 60$  total sectors) of subject  $j$  was defined as:

$$\text{SNR}_{ij} = \text{RMS}_{ij}(45 \text{ to } 150 \text{ ms}) / [\sum_i \text{RMS}_{ij}(325 \text{ to } 430 \text{ ms}) / n] - 1. \quad (1)$$

The denominator in (1) is the average of the individual RMS values for subject  $j$ . An estimate of false positive rates was obtained from the distribution of SNR values for the noise window following Hood et al. (2002):

$$\text{SNR}_{ij} = \text{RMS}_{ij}(325 \text{ to } 430 \text{ ms}) / [\sum_i \text{RMS}_{ij}(325 \text{ to } 430 \text{ ms}) / n] - 1. \quad (2)$$

Thus we obtained 1,080 SNR values (6 subjects  $\times$  3 physical derivations  $\times$  60 locations). An analysis of the distribution of these SNRs showed that SNRs of  $\geq 1.0$  will be part of the noise distribution only with a probability of 1.6% and 0.4% for pattern-onset and pattern-reversal stimulation, respectively. Therefore, a threshold SNR of 1.0 was selected to assess whether a signal is a veridical response.

To quantitatively assess the similarity of the waveforms obtained after pattern-onset and pattern-reversal stimulation, we calculated correlation coefficients of the two mfVEPs for two time windows: (a) in the signal window (i.e., 45-to-150 ms after stimulus onset), and (b) in the noise window (i.e., 325-to-430 ms after stimulus onset). Thus we obtained a pair of correlation coefficients for each visual field location in each subject. These were further evaluated only for visual field locations with suprathreshold responses (SNR > 1.0) to both stimuli. The comparison of these measures allowed us to quantify the similarity of the waveforms after pattern-onset and pattern-reversal stimulation.

To assess whether pattern-onset and pattern-reversal responses depend differently on eccentricity, we calculated the ratio of the responses (reversal/onset response) for each eccentricity and assessed the statistical significance of this dependence using the log ratios. We performed independent univariate ANOVAs for the respective SNR and RMS ratio and the respective electrode location.

## Results

Examples of the responses to pattern-reversal and pattern-onset stimulation are given for two representative subjects in Figure 1. These responses were recorded at the Oz-Iz derivation and are spatially arranged as a re-projection of the signals to the visual field locations that evoked them. The original traces are depicted in A and C. The signal-to-noise-ratio (SNR) for each of these traces is depicted in B and C, where symbol size represents SNR magnitude. A signal drop-out (SNR < 1.0) is indicated by X, thus representing a spurious scotoma. Figure 1 demonstrates two typical features of mfVEP recordings that are evident for both pattern-reversal and pattern-onset stimulation: (1) Great variability of the signal

strength across the visual field and across subjects. At some locations there is a complete signal drop out (SNR < 1.0, indicated by X), which results in spurious scotomata in the reconstructed visual field map. (2) Variability of the shape of the mfVEPs across the visual field. There is a polarity reversal of the signals along the horizontal meridian (i.e., signals above and below the horizontal meridian tend to have an inverted polarity). It should be noted that this polarity reversal is observed for both stimulus conditions and for both subjects presented. Close inspection of Figure 1 shows that this high degree of similarity of the trace shapes between the two stimulus conditions can also be observed at visual field locations other than the horizontal meridian. This is underlined by the correlation of the pattern-onset and pattern-reversal responses for these subjects [correlation coefficients (median) in the signal vs. noise window, respectively; J.K.: .69 vs. .04; I.S.: .72 vs. .02] and across the entire 6 subjects tested [correlation coefficients (median, lower, and upper quartile) in the signal vs. noise window, respectively: .62, .45, .75 vs. .07, -.39, .29]. The data, therefore, indicate a similar topographical distribution of pattern-onset and pattern-reversal responses.

Although similar in shape, pattern-onset and pattern-reversal responses exhibit markedly different eccentricity dependences in their response strength. This feature is demonstrated in Figure 2 and analyzed more formally in Figure 3. In Figure 2, a comparison of the signal strength (SNR) for pattern-reversal stimulation and for pattern-onset stimulation is presented for the same subjects that served as examples in Figure 1. Greater pattern-onset than pattern-reversal responses are indicated by filled symbols, while the inverse relationship is indicated by open symbols. For these illustrations, the most reliable signal (i.e., the signal with the greatest SNR at a specific visual field location) was selected from the corresponding recordings of the six derivations that are obtained after re-referencing the three physical derivations. As is evident from subject J.K., pattern-onset stimulation clearly activates the central visual field (up to 6 or 10°) more strongly than pattern-reversal stimulation, whereas pattern-reversal responses dominate in the periphery. This trend, though less distinct, is also evident from the second example, subject I.S. A quantitative analysis of this feature is given in Figure 3. Here, we depicted the mean pattern-onset response relative to the pattern-reversal response as a function of eccentricity. A ratio smaller than 1.0 indicates greater pattern-onset responses, while a ratio greater than 1.0 indicates greater pattern-reversal responses. It is evident that pattern-onset responses exceed pattern-reversal responses in the central visual field while they are smaller in the periphery. The results reach significance at the derivations OL and Oz, while the trend is also evident for derivation OR (ANOVA for RMS at OL, Oz, and OR:  $p = .0125$ ,  $p = .0029$ , and  $p = .22$ ; ANOVA for SNR at OL, Oz, and OR:  $p = .0003$ ,  $p = 0.0014$ , and  $p = .11$ ).

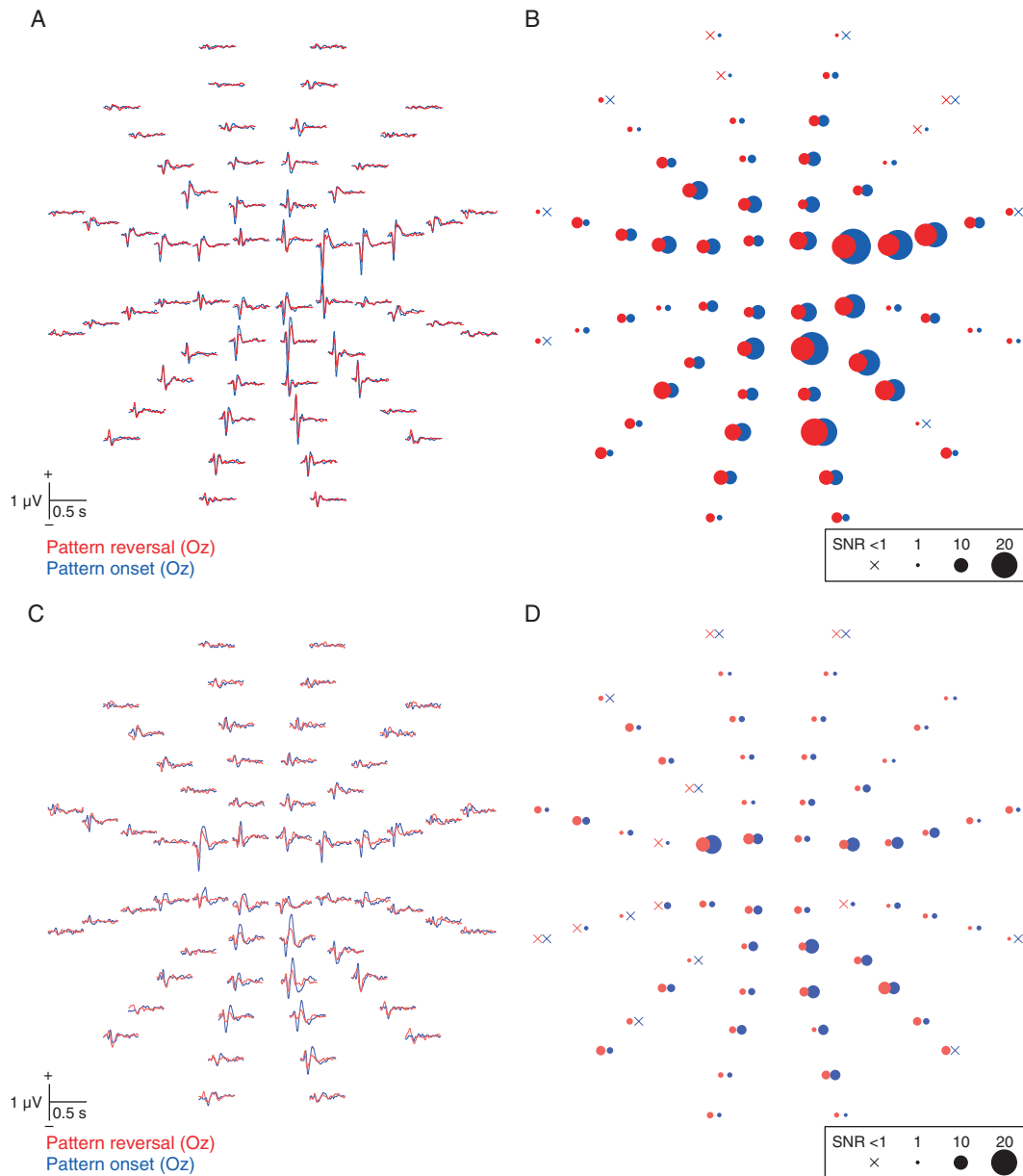


Figure 1. Two representative examples (J.K. and I.S. in panels A, B and C, D, respectively) of mfVEP responses to pattern-reversal (red) and pattern-onset stimulation (blue) as recorded from the Oz-Iz derivation. The panels on the left (A and C; scale bars 1  $\mu$ V and 0.5 s, respectively) depict the original traces while the panels on the right (B and D) depict the resulting SNRs. SNR plots for pattern-onset and pattern-reversal are displaced with respect to each other for clarity. Filled symbols are scaled by SNR, X indicates SNRs < 1.0 (i.e., spurious scotomata). Note the variability of the response strength across the visual field. Pattern-onset responses tend to exceed pattern-reversal responses in the central visual field while this trend is inverted in the peripheral visual field. [Greater responses in the original traces (panels on the left) do not result in greater SNRs for every single visual field location (panels on the right), as the SNR metric depends on the mean response in the noise window (see “Methods”), which can differ between pattern-onset and pattern-reversal responses.] Remarkably, systematic variations of the shape of the traces, especially the polarity reversal along the horizontal meridian, are evident for both pattern-onset and pattern-reversal responses.

It should be noted that pattern-onset mfVEP recordings take twice as long as pattern-reversal mfVEP recordings. The reduction of the recording time for pattern-onset mfVEP by a factor of 2 would yield equal recording times for both stimulus conditions, but is

expected to reduce SNRs for pattern-onset mfVEPs by  $\sqrt{2}$ . Therefore, SNR-ratios given in Figure 3 would have to exceed a value of  $1/\sqrt{2} \approx 0.7$  to indicate the dominance of the pattern-onset response for equal recording times of both stimulus conditions.

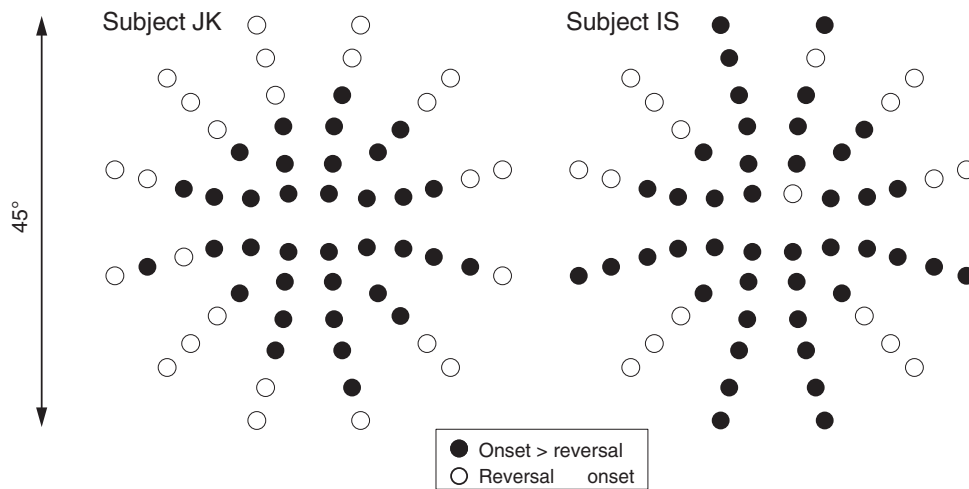


Figure 2. Comparison of pattern-reversal and pattern-onset responses for the two subjects from Figure 1. To evaluate only the most reliable signals, the greatest responses at a given visual field location have been selected from six derivations (three physical derivations and three derivations obtained after re-referencing). Closed symbols (●) indicate that SNRs of pattern-onset responses exceed those of pattern-reversal responses; open symbols (○) indicate the opposite. From both subjects, it is evident that pattern-onset responses exceed pattern-reversal responses in the central 10° of visual field, with an opposite trend in the periphery (10-22°).

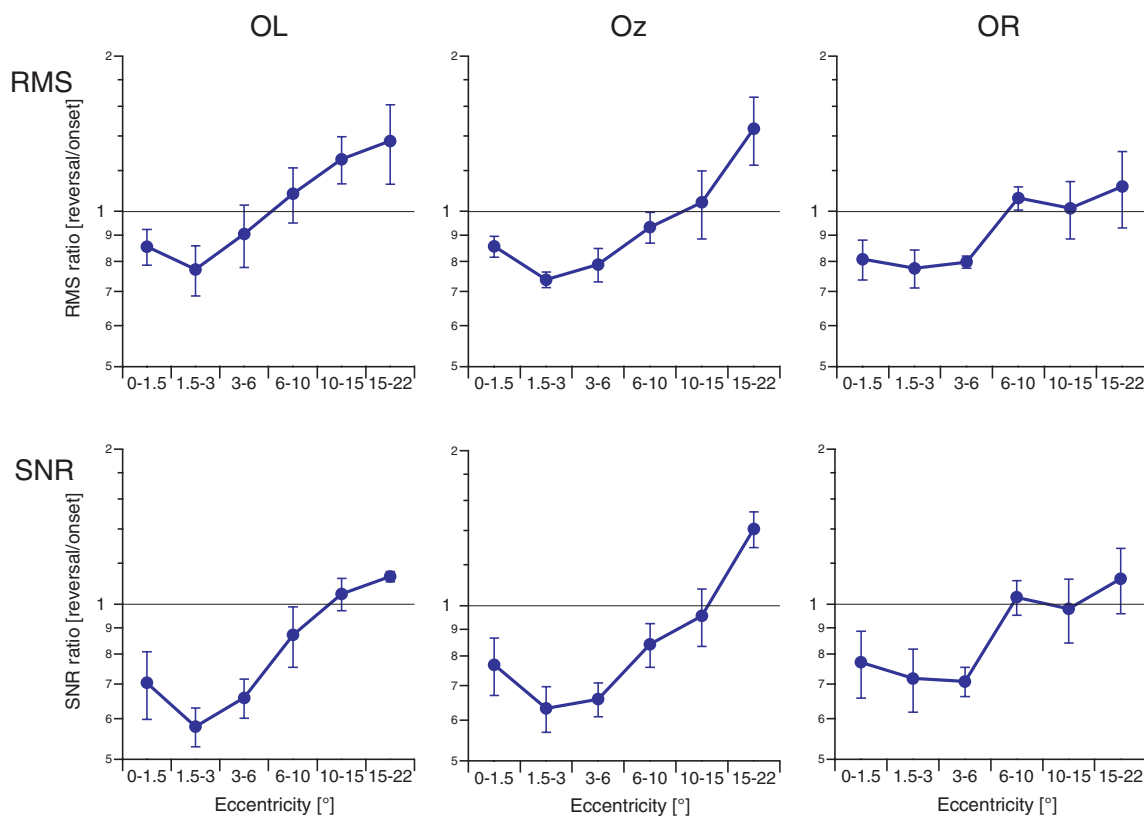


Figure 3. Eccentricity dependence of the pattern-reversal and pattern-onset response across all six subjects ( $\pm$ SEM). The ratio of pattern-reversal/pattern-onset response as recorded from the three physical derivations used is given as a function of eccentricity. RMS-ratios are depicted in the top row, while SNR-ratios are depicted in the bottom row. Only visual field locations that exceeded an SNR-threshold of 1.0 were included in the analysis. A ratio of 1.0 indicates the same response strength for the two stimulus types (solid line), a ratio  $<1.0$  indicates the dominance of the pattern-onset response, and a ratio  $>1.0$  indicates the dominance of the pattern-reversal response. The dominance of the pattern-onset response in the central visual field is evident at all derivations.

Finally, we assessed the practical implications of the increased pattern-onset response in the central visual field and compared the number of false positives (i.e., the number of spurious scotomata obtained after pattern-reversal and after pattern-onset stimulation). As for Figure 2, this analysis is based on the most reliable signals that were obtained at the six derivations after re-referencing. We depicted the number of false positives as a percentage of the visual field locations tested as a function of eccentricity in Figure 4. We pooled the two most central eccentricity rings so that 12 visual field locations contribute to each eccentricity bin. Consequently 100% refers to the entire set of 72 visual field locations in a specific eccentricity bin across subjects (12 visual field locations per eccentricity bin  $\times$  6 subjects). For pattern-reversal stimulation, we found around 2.5% false positives evenly distributed across eccentricities, while we did not find any false positives for pattern-onset stimulation in the central 6° and only 1.4% false positives within the adjacent annulus from 6 to 10°. It must be noted that beyond this eccentricity false positives reach more than 30% for pattern-onset stimulation and therefore clearly exceed the number of false positives observed after pattern reversal stimulation in the periphery. A combination of both stimuli reduced the number of false positives down to less than 1.5% of the entire 60 visual field locations tested.

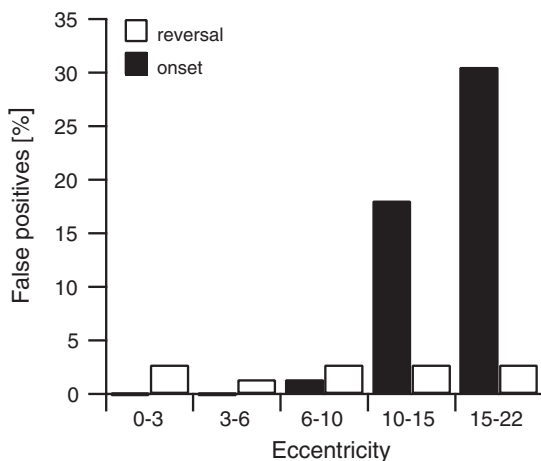


Figure 4. Practical implication of the enhanced pattern-onset mfVEP responses in the central visual field. The proportion of “silent” visual field locations (false positives, SNR < 1.0 at all six derivations,  $n = 6$ ) is given as a function of eccentricity for pattern-reversal (open bars) and pattern-onset responses (filled bars). In the central 10°, pattern-onset responses produce almost no false positives (number of false positives < 0.5%). Consequently, pattern-onset responses have a greater specificity than pattern-reversal responses for the detection of scotomata in the central visual field, an advantage that is lost in the periphery where the number of false positives after pattern-onset stimulation clearly exceeds that after pattern-reversal stimulation.

## Discussion

Our experiments were based on two lines of prior evidence, namely that classical pattern-onset VEP responses can exceed those to pattern-reversal stimulation and that pattern-onset stimulation targets extra-striate in addition to striate cortex. Therefore, we expected to record more reliable responses for pattern-onset than for pattern-reversal stimulation. Furthermore, we expected that pattern-reversal and pattern-onset mfVEPs would arise from different generators, which would therefore result in a different visual field topography of the responses and possibly complementary visual field topographies. We did find pattern-onset responses to exceed those to pattern-reversal, but surprisingly only in the central visual field. Even more surprisingly, we found the topographical distribution of the signals to be remarkably similar for the two stimulus types.

The visual field topography of the pattern-reversal mfVEP has been widely accepted to be a powerful cue to its origin. A remarkable feature of this topography is the polarity reversal of the responses across the horizontal meridian. This polarity reversal is assumed to reflect the convolution of the calcarine sulcus, as the dipoles reverse orientation at this anatomical structure, which should result in an inverted response polarity at the respective derivation (Hood & Greenstein, 2003). Consequently, it has been concluded that the mfVEP originates in the calcarine sulcus, the main location of V1. In fact, V1 has later been confirmed as a generator of the pattern-reversal mfVEP by dipole location studies (Slotnick et al., 1999). In this study, we report the polarity reversal across the horizontal meridian not only for pattern-reversal but also for pattern-onset responses, and therefore conclude that not only the pattern reversal mfVEPs but also the pattern-onset mfVEPs are predominantly generated in V1.

Although pattern-reversal and pattern-onset mfVEPs appear to originate in the same visual area, they differ in response amplitude and their dependence on eccentricity. Two questions arise. Why does pattern-onset stimulation elicit greater responses in the central visual field, and why is this effect restricted to the central visual field? A look at the temporal characteristics of the stimuli used might help to shed light on the former issue. During pattern-reversal stimulation contrast borders are present for the entire stimulation epoch. During pattern-onset stimulation, contrast borders are present for less than half that period (at least one uniform gray frame is interleaved between pattern frames). Contrast adaptation should therefore be expected to affect pattern-reversal responses more severely than pattern-onset responses. Indeed, contrast adaptation is known to reduce the VEP amplitude (Victor, Conte & Purpura, 1997; Heinrich & Bach, 2002). As a consequence, pattern-reversal responses should be reduced due to contrast adaptation more severely than pattern-onset responses. Evidence that this

might be the case is provided in a publication that has appeared during the revision of the present study (James, 2003): Using pattern-onset stimulation in an mfVEP design, he separated pattern-onset pulses by an epoch of at least 460 ms of uniform grey, whereas in the present study this epoch lasted only one or a few multiples of 13 ms. Consequently, the effect of contrast adaptation should be even smaller in James's compared to our study, and indeed he reports pattern-onset response amplitudes that exceed pattern-reversal responses 16-fold.

For the second issue (i.e., the differential effect of eccentricity on pattern-reversal and pattern-onset amplitude), no entirely convincing hypothesis has occurred to us. Pattern-reversal and pattern-onset responses might, to some degree, be associated with different neuronal populations. We can at present only speculate which these might be. Of course, the parvo/magno-dichotomy (P/M) of the visual system comes to mind. Indeed, it has previously been demonstrated that both the P- and the M-system contribute to the mfVEP (Baseler & Sutter, 1997; Klistorner, Crewther & Crewther, 1997). However, a careful evaluation of contrast and chromatic characteristics of pattern-onset responses compared to pattern-reversal responses is necessary to test whether the different eccentricity dependence of pattern-reversal and pattern-onset mfVEPs is related to the P/M-dichotomy of the visual system. Another explanation for the relative decrease of pattern-reversal responses in the central visual field might be the differential effect of fixation instabilities (e.g., microsaccades) on pattern-onset and pattern-reversal responses. The latter are more strongly reduced by fixation instabilities (Saunders, Brown, & McCulloch, 1998). In our experiments, check size increases with eccentricity; the diminishing effect of fixation instabilities on pattern-reversal responses should consequently be expected to level off in the periphery. Therefore, the consideration of fixation instabilities might help to explain the differential effect of eccentricity on pattern-reversal and pattern-onset responses. Further experiments are underway to specifically test this hypothesis.

Although a combination of pattern-reversal and pattern-onset stimulation reduces the number of false positives obtained by mfVEP visual-field perimetry down to less than 1.5%, this approach is probably too time consuming to enter clinical practice. However, any assessment of the central 10° of the visual field might well benefit from the use of pattern-onset stimulation.

## Acknowledgments

Supported by the German Research Council (DFG)HO/2002-3 and BA877-14/1. We thank Daphne McCulloch for helpful comments.

Commercial relationships: none.

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