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BRIEF COMMUNICATION

Examination of short binary sequences for mfERG recording

Richard P. Hagan · Anthony C. Fisher · Malcolm C. Brown

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Abstract The mfERG, when first introduced by Erich Sutter used long sequences with short periods (~15 ms) between steps (flashes). Since then a number of studies have introduced slower or modified sequences to emphasise Oscillatory Potentials (OPs), Optic Nerve Head Components (ONHC) or the s-wave. With this reduction in the rate of presentation many of the investigators have reduced the length of the sequence to allow a shorter recording period. This is also desirable for patient comfort and co-operation in diagnostic investigations. When reducing the length of the sequence there is a risk that it will be too short to ensure orthogonality of the first order component and all significant higher order components, particularly when a large number of areas (hexagons) are stimulated. This paper aims to verify that a short sequence (using the sequence used by the Roland Retiscan® stimulating 19 hexagons) is capable of keeping responses of both first and higher orders separate for each stimulating area. The sequence was investigated by placing photodiodes connected to a Diagnosys Espion® and then exported to Excel® and MATLAB® for analysis. It was determined that the sequences used were *m*-sequences length $n = 9$. The photodiode only responded to flashes of light so was unable to detect a correcting 0 at the end of sequence. The sequences driving each hexagon were then determined and found to be shifted 26 steps from each other. The correlation coefficients between all sequences was found to be $-1/(2^n-1)$. The sequences to decode the second order kernels were determined and the correlation coefficients between each of these sequences, and between these and the original sequences, were also $-1/(2^n-1)$. This work provides a mathematical validation of the use of short sequences for slow mfERG, and describes an empirical test method.

Keywords Multi-focal electroretinogram · mfERG · *m*-Sequence · Short sequence · Retiscan® · First order kernel · Second order kernel

R. P. Hagan (✉) · A. C. Fisher · M. C. Brown
Clinical Engineering Department, Duncan Building, Royal Liverpool University Hospital, Prescot Street, Liverpool L9 8XP, UK
e-mail: R.P.Hagan@liv.ac.uk

Introduction

In 1991 Sutter [1] introduced the use of *m*-sequences to code ERG stimulation and to decode electrical responses taken simultaneously from several discrete areas of the retina. This technique had the great advantage of collecting retinal responses from many areas over a short period of time. In an early demonstration [2] he stimulated 243 hexagonal segments using a 16 bit *m*-sequence

(65,535 steps long). The multi-focal ERG has proved to be a useful clinical tool. Responses have been reported to be reduced and delayed in RP [3, 4] and its central segment reduced in Stargardt macular dystrophy [5], in occult macular dystrophy [6] and AMD [7]. It was noted early on that the waveform shape from the mfERG was different to that from full field (slow) stimuli, and in particular the response from a retina with arterial occlusion [3] had a different morphology casting some doubt as to the origin of its waveforms. Hood et al. [8] slowed the mfERG stimulus down with seven blank (these blank frames had a luminance to match the background light in the ISCEV photopic single flash) filler frames (CRT update 75 Hz). The resulting waveform had a morphology which closely matched the full field photopic ERG complete with oscillatory potentials (OPs). Previous to this Wu and Sutter [9] had slowed the mfERG to elicit OPs from the human retina by introducing three filler frames, and subsequently OPs have been used in the differential diagnosis of complete and incomplete congenital stationary night blindness CSNB [10] again with three filler frames. mfERG OPs were found to be reduced in diabetes with the use of seven filler frames [11]. All the above used CRT stimulation with 75 Hz frame rate and VERIS software. In 1999 Sutter et al. [12] identified an optic nerve head component within the mfERG, though it was small in amplitude. Sutter et al. [13, 14] and Shimada et al. [15] optimised the multi-focal stimuli to recover the ONHC by introducing a blank, full field flash and blank between steps. Fortune et al. [16] used this optimised stimulating protocol to investigate changes in glaucoma.

Sano et al. [17] also reduced the rate of the mfERG stimulus to help optimise the recording of the s-wave (a small positive component on the descending limb of the main positivity), which is thought to reflect ganglion cell activity.

Contributions to the mfERG from monkey retina have been investigated with pharmacologic agents to both fast [18, 19] and slow stimuli [20]. To facilitate the slow stimulus (14 blank filler frames), a shortened *m*-sequence was used on the VERIS of 2^{11}–1 length (2047 steps), which required a minimum recording period of 7 min before signals from individual hexagons could be calculated. This study suggested similar origins of the negative and positive waves of the mfERG as for the full field photopic ERG.

This study looks at a system using short sequences running a relatively long base period (bp) (83 ms ~5 CRT frames). The base period is the period between successive steps of the binary sequences and in most published work the bp has been one frame update period, typically 13–17 ms (see Fig. 1 for illustrations). When using a CRT stimulator, bps of integer multiples of the frame update period are the most convenient. When applying the slow stimulus a shorter *m*-sequence has been applied to keep recording times down [8, 17, 20].

Care is required when selecting and decimating a shorter sequence to ensure orthogonality of hexagon responses to each other and to higher order responses bleeding in from a different hexagons.

To successfully encode information for a given number of stimulated areas, the length of the 'mother' sequence must exceed first the number of areas. The non-linear nature of the retinal response to rapid flash stimulation requires that higher order responses must be considered. If we consider here only the effects of an immediate preceding flash on the response (second order kernel, first slice—SOK_1), then the SOK_1 decoding sequences cannot be used for stimulating any hexagon (for a full description of the multi-focal higher order kernels see Sutter [21]). Thus, the minimum theoretical number of steps in the sequence is twice the number of stimulated areas. Should significant higher order kernels exist, the sequence length must again be increased.

A further consideration in multi-focal stimulus design is the bp of the stimulus. In general, a longer bp results in less influence on the responses due to preceding flashes, rendering the higher order effects less significant [22].

Having a recording epoch longer than the bp will require expansion of the *mother sequence* to create more 'unused' sequences (i.e. not used to control a stimulated segment). However, a rigid method of decimation is not mandatory and one may find having first order stimulus sequences equally spaced does not necessarily result in the

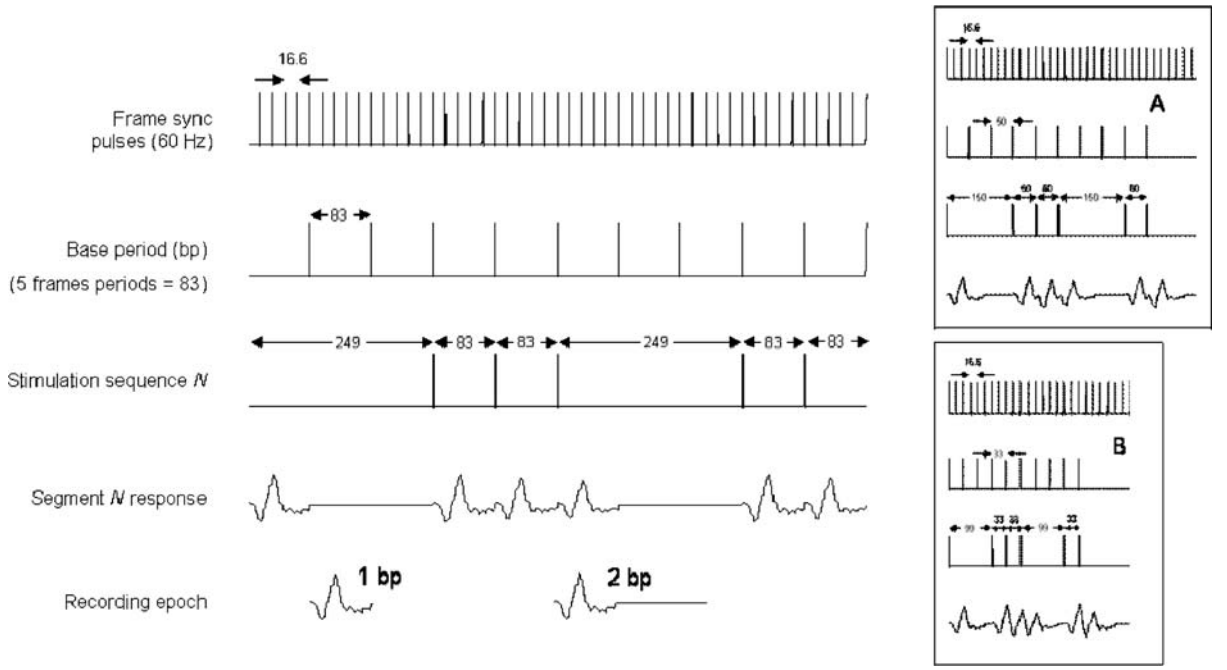

Fig. 1 Example timing relationships between frame rate (fr), base period (5 × fr), the stimulation sequence N and the retinal response from its corresponding segment (times are ms based on 60 Hz frame rate). Insets A and B show the effects of decreasing the base period (50 and 33 respectively): the individual retinal responses overlap

sequences determining the higher order kernels being optimally placed to avoid clashes.

All of these factors must be considered before deciding whether a sequence is valid for the recording requirements. Pseudo random binary sequences (PRBS) in the form of *m*-sequences have useful properties for mfERG. These are an odd number in length (2^n–1) causing a non zero correlation between consecutive sequences of –1/(2^n–1) (see Ireland et al. [23] for fuller exploration of *m*-sequences and their properties). The present study uses as its example short sequences as used by the Retiscan® (Roland Consult, Brandenburg, Germany) to determine if they have the properties of *m*-sequences and if mathematically they could keep responses and higher order effects from separate hexagons orthogonal.

Methods

The Retiscan was operated using 19 hexagons in its default stimulation and recording mode (83 ms bp —5 frame periods, 83 ms recording epoch). In this mode the first CRT frame is either on or off depending on the sequence, and the next four are always blank. Since the stimulus is provided from a CRT, the 'on frame' is only white for 1 or 2 ms, depending on the phosphor. A photodiode was placed on hexagon 1 and a second placed sequentially on hexagon 2–19 (see Fig. 2). This allowed the stimulating sequence to be determined for each hexagon and its starting time relative to the start of the sequence for hexagon 19 (taken as the *mother sequence*). The outputs of the diodes were recorded on an Espion® (Diagnosys UK Ltd., Cambridge) and exported as CSV files for further analysis. Within MS Excel®, a macro was written to identify leading edges of the photodiode response (see Fig. 3). This involved setting a threshold value to determine when the recorded spikes were significant and forcing significant spikes to be more than one data point apart (2 ms). A vector containing latencies (ms) of when the photodiode crossed the threshold was created and time differences between flashes calculated. Using MATLAB it was possible to assign a binary code to every time interval

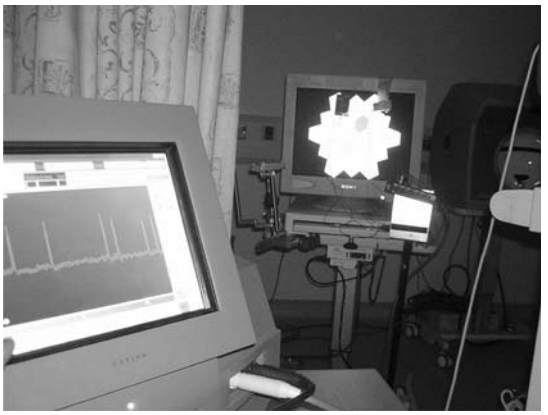

Fig. 2 Set up for recording the sequences. Nineteen segment display with photodiodes (right) and flash sequence captured (left)

between flashes i.e. 83 ms = 1, 166 ms = 10, 249 ms = 100 etc. This produced the binary column vector of the sequence being recorded. All subsequent analysis was performed in MATLAB® R13 (Mathworks, Inc, Natick, MA, USA. When all sequences were determined, 0's were replaced with –1's and the correlation coefficients were calculated for each of the 19 stimulating segments with respect to all others. Using modulo 2 arithmetic, the second order sequences were derived (see Sutter [21] for fuller description). The correlation coefficients were calculated between the second order sequences, and between these sequences and all first order sequences.

Results

From the photodiode output it was determined that the sequence tested by the Retiscan had the properties of an *m*-sequence. The sequence itself was one that could be generated from a 9 long shift register (that is 511 long or 2^9–1). Each hexagon was driven by the same sequence, but shifted by 26 steps, which is the nearest integer to 511/19 (rounded down): thus, the first order kernel (FOK) sequences were maximally separated. It was found that all the sequences generated from the *mother sequence* had correlation coefficients of –1/511. The sequences recovering the SOKs were found to have the same correlation coefficients with each other and with the stimulating sequence. Of interest, is that the sequence for SOK_1 of hexagon 6 was only shifted by one element from the FOK sequence for hexagon 19 etc. (see Table 1). This has the potential to allow bleed from the SOK_1 of hexagon 14 into the FOK of hexagon 19 if the recording epoch is longer than the bp (see Fig. 4). A further problem in keeping responses separate is that of the induced component. The induced component in the SOK of hexagon 14 is the contribution from the preceding FOK of hexagon 19 and in the diagrammatical representation it can be seen will make quite an impact on the recovered SOK of hexagon14. The reason that this induced component is potentially troublesome is that the response epoch is may be longer than the recording epoch considered here. The Roland uses quite a

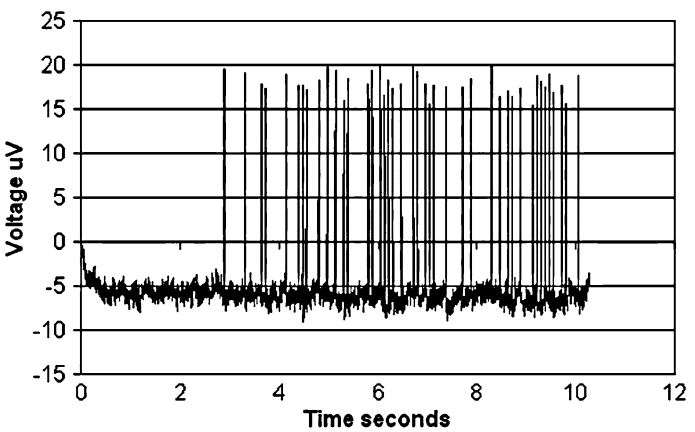

Fig. 3 Photodiode example response from hexagon 1

Table 1 Showing the starting point of the *m*-sequence for each hexagon FOK (relative to hexagon 19) and the derived SOK, this illustrates the relative closeness of FOK and SOK in several hexagons (e.g., hexagon 14's SOK is one step after hexagon 19's)

Hexagon	FOK	SOK
19	1	383
18	27	409
17	53	435
16	79	461
15	105	487
14	131	2
13	157	28
12	183	54
11	209	80
10	235	106
9	261	132
8	287	158
7	313	184
6	339	210
5	365	236
4	391	262
3	417	288
2	443	314
1	469	340

different (double flash) paradigm to record SOK, though this is beyond the scope of this paper. There was a 1.9 s period of stimulation before recording began, which we assume is to precondition the retina. This all results in a nominal recording period of approximately 46 s for a single sequence.

Since the photodiode can only respond when a flash occurs, it was not possible to determine whether the last step in every stimulating sequence was a zero (blank screen) as detailed in Roland's literature, which means we cannot say whether an extra blank element has been included, or that the sequence has finished. However, using the same cross-correlation tools as above we calculated that if a 'correcting' step was present the correlation coefficients would all be zero.

Discussion

It has been found that the Retiscan default mode for 19 hexagon stimulus (511 short sequence) uses near orthogonal stimulating (first order) and second order kernel sequences. A small $-1/(2^9-1)$ correlation coefficient remains, as expected. In theory this residual can be eliminated, if need be, by the addition of a one step extension to the individual stimulating and decoding sequences (not the *mother sequence*). Adding a blank step to all sequences at the end, making them 2^n long, and having an equal number of 0's and 1's forces the correlation coefficients to become zero. However, if the final step for all sequences is 0 (i.e. no flash), then given the slow stimulus rate presented here the physiological contribution would be small (only that remaining from a previous flash 83 ms ago from about half of the hexagons).

In an authoritative treatise on the binary kernels arising from non-linearity of the retinal responses, Sutter [21] emphasised the advantages of long sequences and also explained the problems of contamination due to preceding and succeeding stimuli, and their higher order kernels. However, these problems arise in part due to the use of higher stimulation rates, and recording epochs which cover several bps; conditions which are avoided here.

A longer bp, such as one comparable with the response epoch reduces the interaction between sequential responses, therefore minimising higher order kernels. By keeping the recording epoch to one bp, induced components due to the subsequent flash responses in the same hexagon are avoided. The short sequence here allows a full test to be run in only 46 s allowing the operator to stop, repeat, average the repeats, and cease recording when a sufficient SNR has been achieved or indeed the patient can no longer tolerate the test. With advances in computer mathematics, new possibilities are emerging for rapid multi-channel analysis, such as described by James [24] for mfVEP, which uses multiple regression in place of cross correlation.

Conclusion

Using short binary sequences for mfERG can not produce the SNR possible with very long sequences. However, the present examination demonstrates that the necessary mathematical

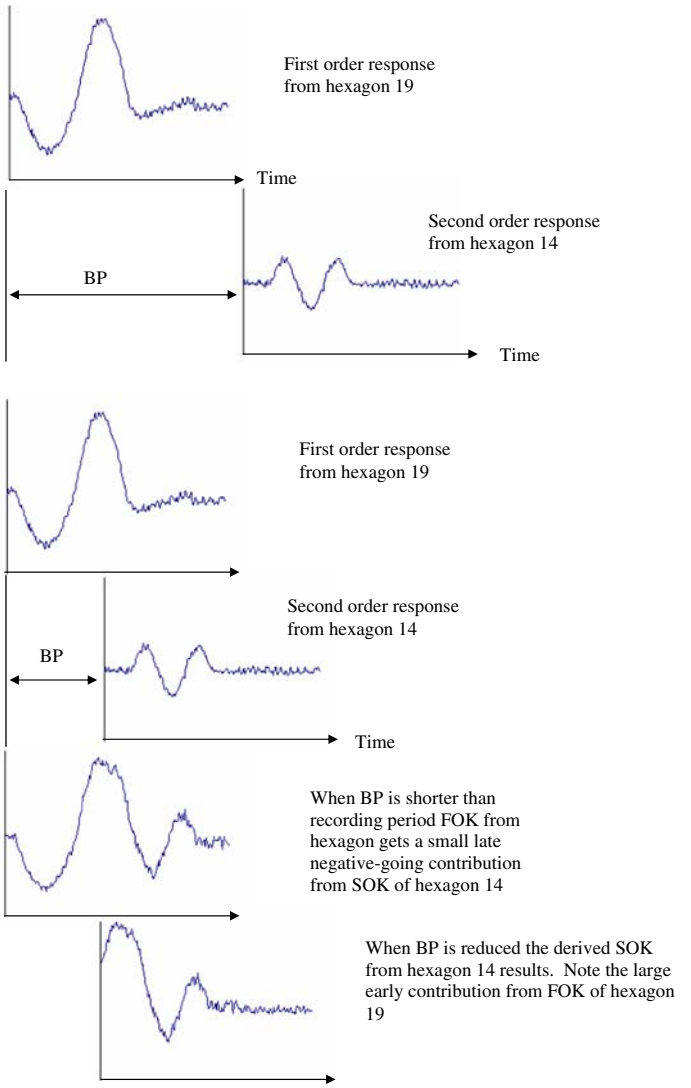

Fig. 4 Schematic showing that the same *m*-sequence will recover the FOK of hexagon 19 and SOK of hexagon 14 and unless separated by enough steps in the *m*-sequence these responses may bleed into the recording window

requirements for reliable mfERG can still be met. Some problems of contamination of the responses can be avoided by using longer base periods with recording epoch no longer than the base period.

Further, in the clinical setting, where responses may be small and noisy, the problems of patient co-operation, muscle noise, light scatter in the eye, fatigue, fixation control, blink artefact handling etc., may be more significant factors in the choice of method than some of the more frequently highlighted technical and mathematical issues.

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