A New ERG Paradigm to Evoke S-cone Potentials

Schatz A. , Wilke R. , Messias A. , Strasser T. , Jägle H. , Gekeler F. , Zrenner E.

Centre for Ophthalmology, University of Tübingen, Germany ;

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Purpose

The blue cone system (S-cone) is difficult to stimulate seperately and elicit low amplitudes with slow latencies in the ERG. Frequently the S-cone response is overlaid of responses originating from middle- and long-wavelength cones (M-/L-cones). To avoid any influences from M-/L-cones in S-cone ERG two special techniques were utilized so far - the silent-substitution-technique (SST) and the selective-adaptation-technique (SAT). The SST is complex for clinical routine measurements. The SAT on the other hand requires a lot of time to saturate the M- and L-cones. The aim is to develop an ERG stimulation technique to recognize the S-cone response in clinical routine examinations and in the same step to assess L-/M-cone function.

Methods

A new triple-flash ERG paradigm was developed by using theoretical and experimental explorations. Physiological characteristics of photoreceptor systems were taken into account for optimizing S-cone stimulation.

Two high intense flashes (red LED 635 nm) were generated at the beginning of the stimulation to saturate the middle and long wavelength cones. A dim blue light was produced thereafter in a constant time by a blue LED (470 nm) to elicit a S-cone response. To potentiate the saturation of green and red cones and to ensure the light adaptation of the rods, an amber high intense background illumination (90 cd/m²) was adhered during the entire measurement.
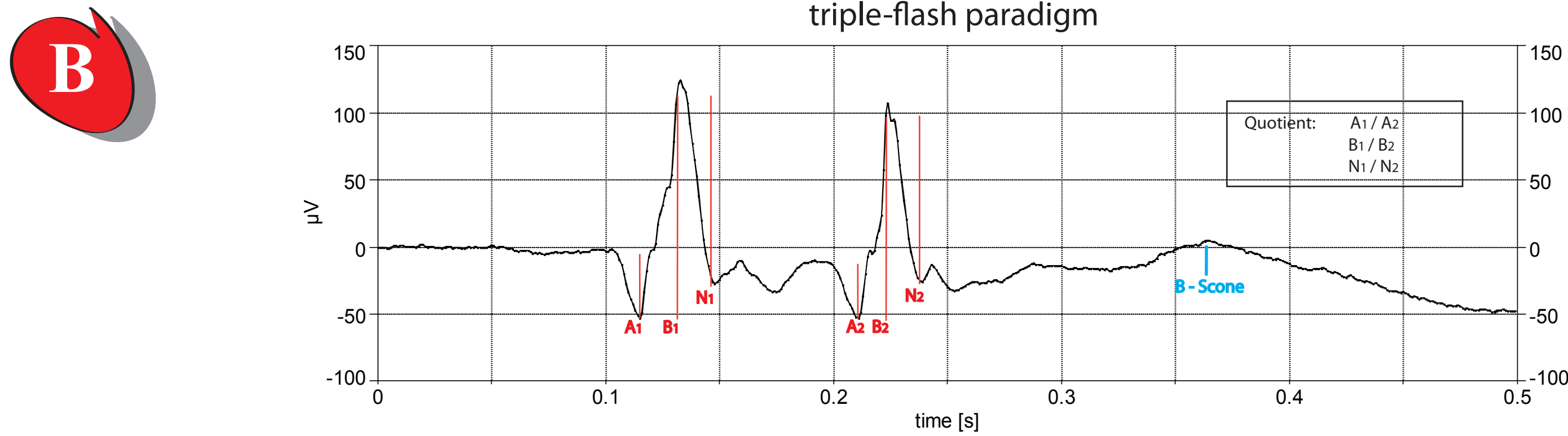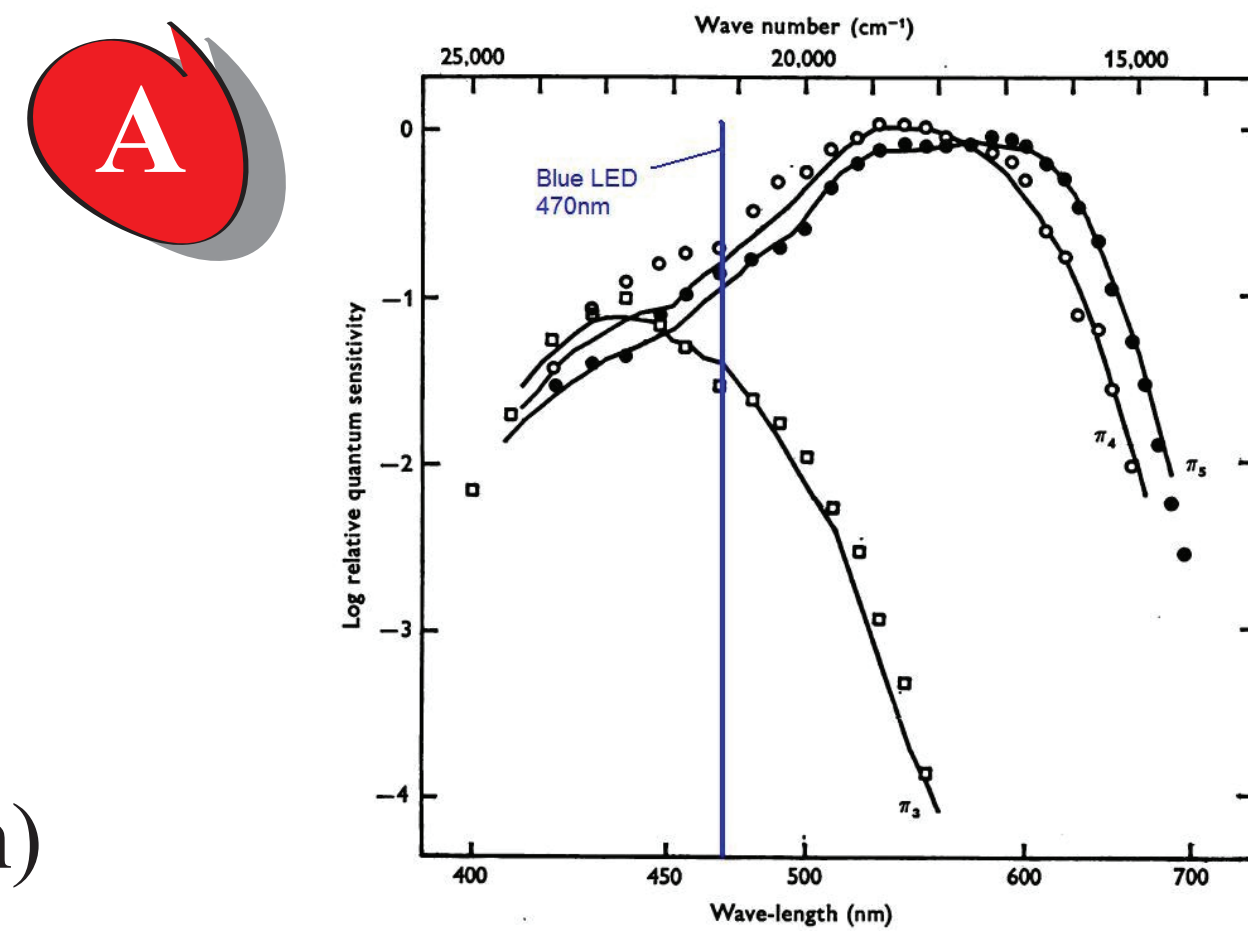
ERGs were recorded in 8 volunteers to identify optimal stimulation settings. Experimental procedure was performed following the declaration of Helsinki. Intensity series were investigated to isolate the ideal stimulus settings for S-cone recording. Different stimulation colors were used (white 6500K, green LED 511 nm, red LED 635 nm) and compared with blue stimulation. ERG components of both saturating flashes and of s-cone responses were analyzed.

Figure 1.:

A.) Spectral sensitivity of cones in accordance to Cavonius and Estévez "Contrast sensitivity of individual colour mechanisms of human vision" in Journal of Physiology 1975 (vol. 248, p. 649-662) with blue LED wavelength used in the study.
B.) ERG curve of triple-flash paradigm in one eye. Analyzed waves (A, B, PhNR, B-Scone) and quotients were marked in the curve.

Results 1

Both saturation flashes were performed with an maximal intensity of 4 phot cd.s/m² and durations of 4 msec. Short durations for the saturation flashes allows the recording of i-waves and PhNR.

It has been identified that ERG potentials derived by the second saturation flash were smaller comparing to the first flash. A quotient of ERG potentials was calculated to describe the presumably time-dependent interrelation between both saturation flashes.
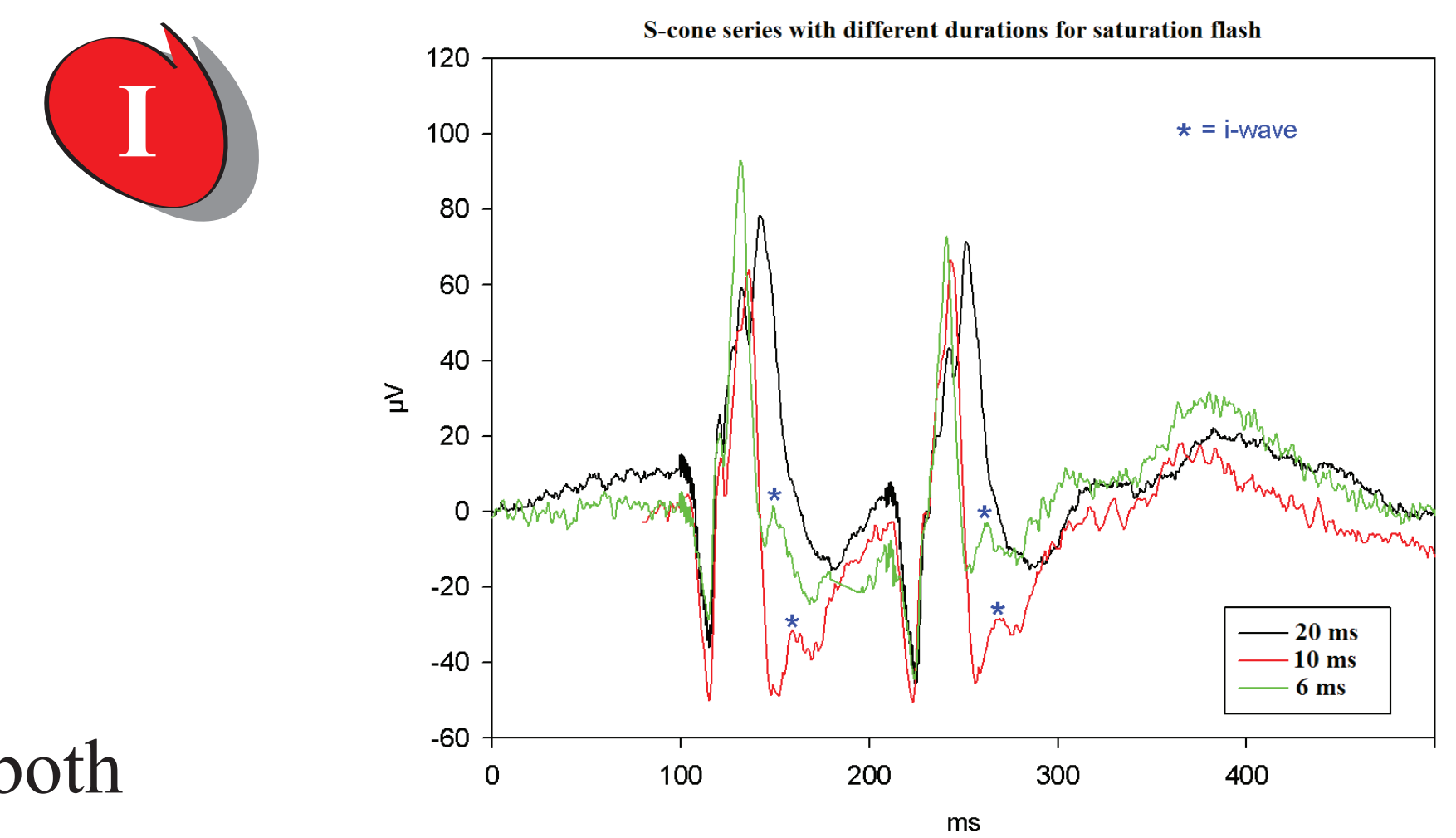
Nr.	Mean (A1)	Std.-Dev.(A1)	Mean (B1)	Std.-Dev.(B1)	Mean (N1)	Std.-Dev. (N1)
1	38,058	3,807	187,156	10,649	177,469	10,982
3	36,263	4,440	228,861	25,156	233,252	29,223
5	24,86	2,3	119,322	16,132	111,75	13,569
6	39,37	8,088	195,539	6,2957	185,265	7,317
8	27,894	3,212	135,050	7,8133	116,708	6,804
9	27,333	5,711	109,294	15,295	102,087	13,56
12	65,028	8,296	253,322	40,75	214,486	43,316
15	40,723	3,794	172,139	17,953	155,188	16,223

Nr.	Mean (A2)	Std.-Dev.(A2)	Mean (B2)	Std.-Dev.(B2)	Mean (N2)	Std.-Dev. (N2)
1	41,623	3,766	174,616	11,12	154,23	9,668
3	40,931	4,927	211,887	23,816	204,089	24,03
5	32,862	2,398	119,579	13,752	97,539	7,143
6	43,12	5,798	181,162	9,212	164,45	5,873
8	27,567	3,313	122,734	7,191	103,101	4,763
9	23,644	3,043	99,382	14,753	89,967	14,25
12	62,942	7,804	235,402	36,677	192,846	40,428
15	42,037	5,422	160,816	13,082	130,688	15,877

	Ratio (A1/A2)	Ratio (B1/B2)	Ratio (N1/N2)
Mean	0,973	1,072	1,143
Std.-Dev.	0,127	0,033	0,022

The third flash for S-cone stimulation was generated for 100 msec. Assuming that Off-responses in ERG are produced by L/M-cones under long-lasting stimulation, the absence of Off-responses is of high importance to verify the disturbance of L/M-cones to developed S-cone protocol.

From measurements in volunteers (mean b-wave amplitude 22.03µV, mean latency 51.54ms) an optimal intensity of 6 phot cd/m2 was derived.
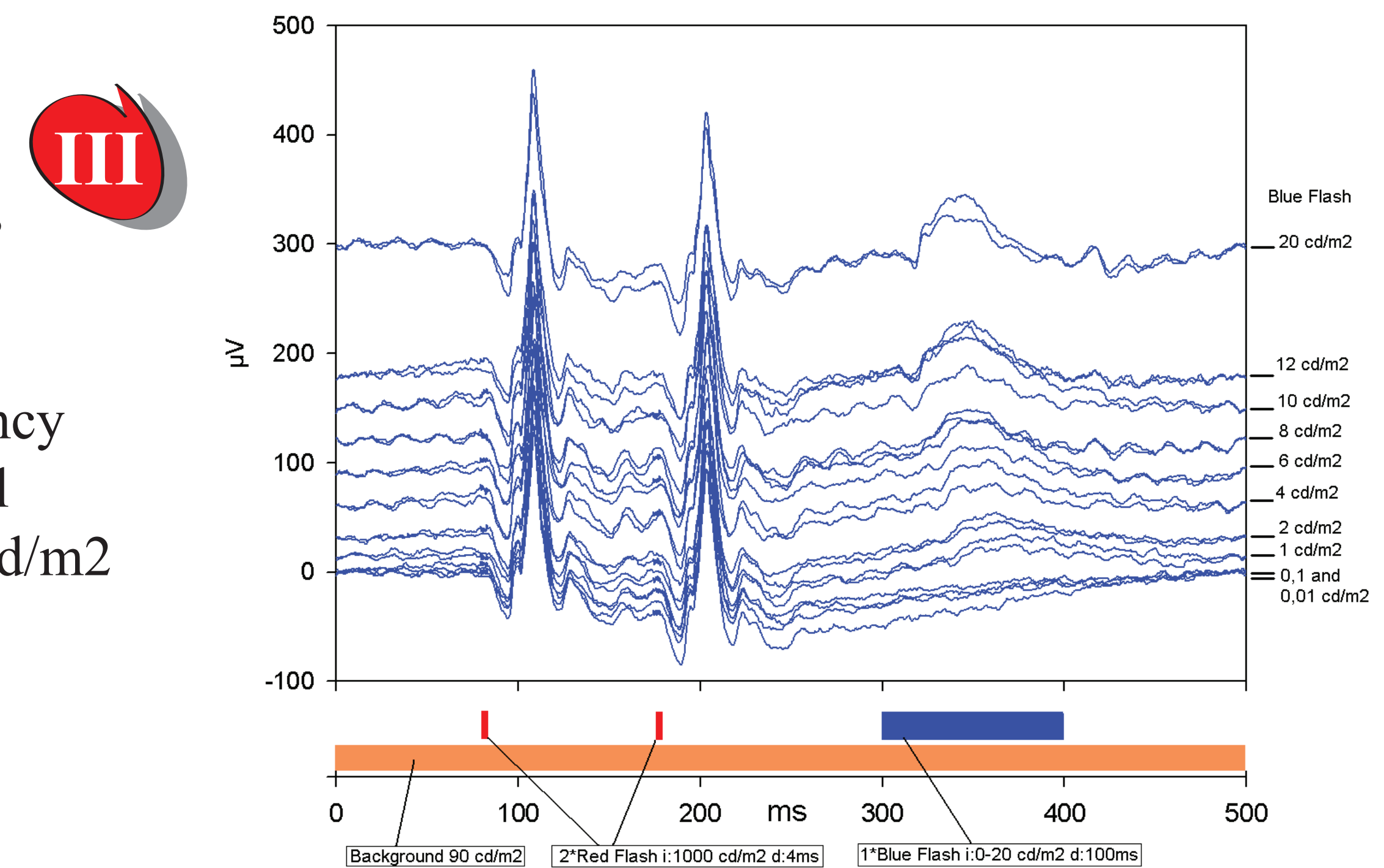
Figure 2.:

I.) Triple-flash stimulation with different (20ms, 10ms and 6ms) durations for the red saturation flashes.
II.) ERG potentials of the first and the second saturation flash as mean of 15 measurements. Table below shows a grand average of quotients between first and second flash.
III.) Intensity series for S-cone stimulation with raising intensities from 0,01 to 20 phot cd/m².
IV.) Usage of different colors instead of blue LED for S-cone stimulation (3rd flash).
V.) Comparison of ERG components derived with different colors.

Results 2

In contrast to the blue LED for S-cone stimulation the red, green and white flashes produced typical ERG properties of L- and M-cones.
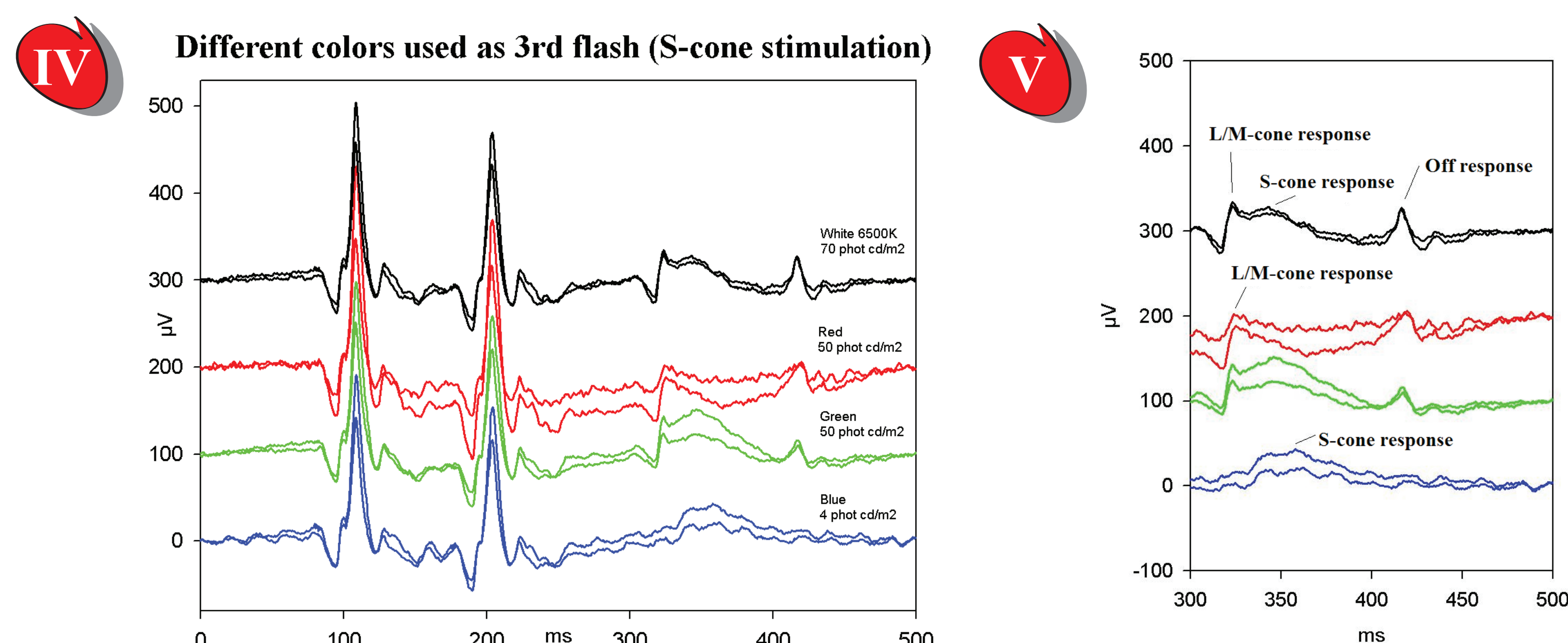
Conclusion

A new triple-flash-technique was developed for S-cone recording. Simultaneously double flash adaptation kinetics in L- and M-cone responses can be evaluated. Thus eye diseases can be assessed by a single photopic ERG protocol, such as vitamin A deficiency, cone-dystrophies and blue cone monochromatism. The s-cone potential can be assessed without a pre-illumination time to separate the M-/L-cone influence; therefore it can be integrated easily in routine clinical ERG examinations.