VEPs IN RESPONSE TO SELECTIVE SHORT-WAVE CONE PATHWAY STIMULATIONS IN HYPOTHYROIDISM

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Abstract: Hypofunction of the thyroid gland (hypothyroidism) is a condition of reduced or missing synthesis of thyroid hormones. Hypothyroidism has an increasing prevalence and therefore is a socially significant problem for both affected individuals and the society. It is well known that thyroid hormones play an important role in the functioning of the nervous system. The lack or reduced amount of thyroid hormones could cause damages in the sensory systems. However, the study of visual deficits in people with hypothyroidism is extremely rare in the literature, probably because the general symptoms of hypothyroidism are severe and hormone replacement therapy is started before vision changes caused by thyroid hormone deficiency manifest themselves.
The aim of the present study was to search for a suitable electrophysiological approach to study the potential disturbances in the visual system of patients with an acquired primary hypothyroidism. We hypothesized that it is most likely to find potential visual malfunctions caused by hypothyroidism in the blue-yellow colour vision system, which is known to be more vulnerable in a number of diseases.
Two groups of participants took part in the study – a group with hypothyroidism and the control group without thyroid dysfunction. Electrophysiological responses were obtained in two different conditions. In the first part of the experiments we registered visually evoked potentials (VEPs) during the above-threshold blue increment and decrement stimulation presented on a bright yellow background in order to selectively stimulate the short-wave cone pathway. The results showed delayed VEP latency in response to both blue increments as well as decrements in hypothyroidism. In the second part of the experiments the VEPs were recorded in response to isoluminant stimuli, appearing at 20° eccentricity along the temporal retinal meridian. The responses showed shorter latency and greater amplitude of the first negative wave recorded to "red" stimuli while this wave was the smallest and with longer latency to "yellow" stimuli and with intermediate values – for "blue" stimuli. The results obtained show a delay in visual information processing in hypothyroidism. Although promising results of the modified two-colour threshold method of Stiles as well as of the silent substitution method, both approaches suffer from certain limitations and we suggest further improvements.

Keywords: VEPs, Colour vision, Hypothyroidism.

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1. INTRODUCTION

Hypothyroidism is a global health problem that can significantly influence well-being (Taylor et al., 2018). Hypothyroidism denotes the hypofunction of the thyroid gland when the synthesis of thyroid hormones is reduced or absent. Reduction of thyroid hormone causes damage to the nervous system because they play an important role in its development. In particular, it has been found in animal models that thyroid hormones play a very important role in the development of the retina (Harpavat & Cepko, 2003). Based on the results obtained Boyes et al. (2018) even suggested that moderate levels of pre-and post-natal thyroid hormones insufficiency modify the visual function of adult rats at both retinal and visual cortex sites of dysfunction.

Nevertheless, the study of visual deficits in people with hypothyroidism is extremely rare in the literature, probably because the general symptoms of hypothyroidism are severe and hormone replacement therapy is started before vision changes caused by thyroid hormone deficiency manifest themselves. Simic et al. (2013) found that various visual-spatial functions in congenital hypothyroidism were affected resulting in impaired ability to integrate parts of the image into a single whole. A delay in the visual information processing rate was reported (Holder & Gondon, 1989; Jaiswal, 2016), reflected in longer latency of the cortical visually evoked potentials (VEPs) during the presentation of achromatic stimuli as well as reduced VEP amplitude. A reduced critical frequency of flicker fusion was reported (Dietzel et al., 2012) and prolonged response time in hypothyroidism (Jaisval, 2016; Vedavathi, 2013). It was found that thyroid hormones in rats are involved in the synthesis of the opsin, the visual pigment, which is synthesized in cones and mediates colour vision both during perinatal development and throughout the individual's life (Glaschke et al., 2011). Therefore, one can expect that the deficiency of thyroid hormones will affect colour vision. Nonetheless, until recently there was very few data on colour vision changes in patients with acquired primary hypothyroidism. Cakir et al. (2015) found diminished contrast sensitivity in hypothyroidism. Recent studies showed altered spatial summation of S-cone chromatic mechanisms (Racheva et al., 2019) and worse colour discrimination along the blue-yellow (B-Y) axis in hypothyroid patients (Racheva et al., 2020).
Three types of cones, short-wavelength (S-cones), middle-wavelength (M-cones) and long-wavelength (L-cones) are involved in the human daylight vision. The ganglion cell that receive input from the M- and L-cones have approximately equal density, although large individual differences. ON cells transmit the signal for luminance increments and OFF cells – the signal for luminance decrements (Wässle & Boycott, 1991). However, S-cone OFF ganglion cells are less numerous, and this morphological result found a psychophysical correlate in the larger area of spatial summation for S-cone decrements (Vassilev et al., 2003). We hypothesized that it is most likely to find potential visual malfunctions caused by hypothyroidism in the blue-yellow colour vision system, because it is more vulnerable in a number of diseases.
The aim of the present study was to search for the suitable electrophysiological approach to study the potential disturbances in the colour visual system of patients with hypothyroidism.

2. MATERIALS AND METHODS

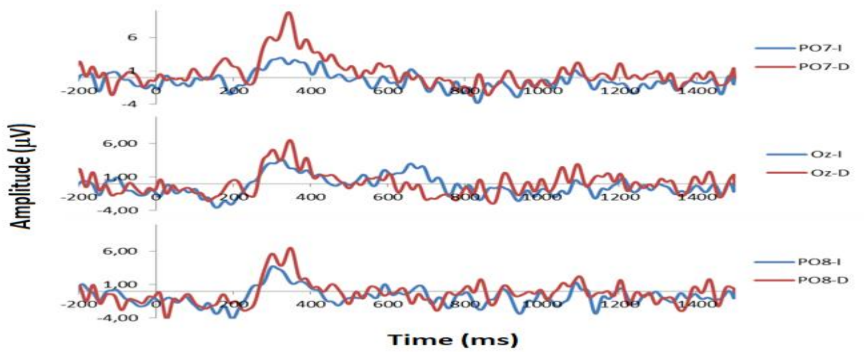
Two experiments with different stimulation conditions were performed in the present study. In the first experiment visually evoked potentials (VEPs) were recorded in conditions of a modified two-colour threshold method of Stiles. The methodological approach of the short-wavelength (S-) cones isolation is described in detail in Vassilev et al. (2000; 2003). The background consisted of bright yellow light (360 cd/m²) and very low blue light (1.3 cd/m²). The spectrum of blue light is close to the spectral sensitivity curve of S-cones. Because all photoreceptors are sensitive to blue light, the yellow background aims to reduce the sensitivity of M- and L- cones and rods so that stimuli are effective only for the S-cones. Test stimuli encompassed the whole screen and consisted of blue light increments or decrements. The monitors size was 29° horizontally and 21° vertically from a distance of 57 cm. The bright yellow component of the background in the experiment was provided by a slide projector. The two light sources were superimposed in front of the subject's eye through a semitransparent mirror. The duration of the stimuli was 2000 ms and their contrast – 10 times above the threshold.
In the second experiment another approach for isolated stimulation of the colour pathways was applied – the silent substitution method using isoluminant stimulation. In this approach, the spectral composition and intensity of the blue, green and red components change so that the colour of the stimulus changes without changing its brightness. The colour should be changed in such a way that the amount of quanta absorbed changes only for one type of cones, e.g. S-cones and their pathways at the level of opponent mechanisms, but remains constant for M- and L-cones along the L-M opponent axis. The chromaticity was determined using modulation from an achromatic background to 90º, 270º, 0º and 180º in the colour space of Derrington, Krauskopf & Lenie (Derrington et al., 1984). This method is described in detail in Zlatkova et al. (2021). Stimuli selectively modulate a particular chromatic mechanism keeping the others silent (silent substitution method). The stimuli were isoluminant colour spots with 4 colours loosely named “blue”, “yellow”, “red” and “green”. Their diameter was 4° from 57 cm and they were presented at 20° in the temporal retina. The observation was performed with the right eye with natural pupil. The duration of the stimuli was 100 ms. The blue and yellow stimuli were presented in one series, and the red and green stimuli – in another.
VEP were recorded from positions Oz, PO7 и PO8 according to the 10-20 system by a BIOPAC system MP100WSW connected to a microcomputer. The ground electrode was positioned on Fp. The reference electrodes were positioned on the left mastoid and the right mastoid. The signal was amplified, band-pass filtered (1–100 Hz) and fed into a microcomputer at a sampling interval of 2 ms. Sweeps started 500 ms before grating onset and lasted for 3000 ms. Traces with artefacts were automatically discarded. The number of sweeps recorded and averaged in

each condition was between 50–100 and depended on the signal-to-noise level expected from pilot experiments. The records were off-line band-stop filtered at 50 Hz.
10 participants separated into two groups took part in the study: a group with hypothyroidism and a control group. The near and far visual acuity of the participants was normal or corrected to normal.

3. RESULTS

The results obtained show that the VEPs from positions PO7, Oz, PO8, registered in response to increment and decrement stimuli in blue-on-yellow selective S-cones stimulation (Figure 1) have clearly differentiated negative-positive complex around 200 ms. This complex reflects the response to the stimulus presentation. The responses to decrement stimuli have a greater amplitude and longer latency than the responses to increment stimuli, an asymmetry which also appeared in the VEPs in hypothyroidism.

Figure 1. Example of the VEPs registered in response to increment (I) and decrement (D) stimuli in blue-on-yellow selective S-cones stimulation. Data of one participant from the control group.



The VEPs of the participants from the both groups however, are very "noisy" as illustrated on Figure 1. Probably this is due to the work of the slide projector which provided the bright yellow component of the background necessary for the S-cones selective stimulation. The low signal-to-noise ratio in the VEPs recorded in these conditions made the comparison of the recordings of the two groups, with hypothyroidism and the control group, difficult and unreliable. In order to increase the signal-to-noise ratio and to attenuate the contribution from remote current generators, we applied the Laplacian analysis. The three-point Laplacian was calculated off-line as twice the potential at Oz minus the sum of the potentials at the lateral electrodes (Mihaylova et al., 1999).

Figure 2. Example of the Laplacians obtained in response to increment (I) and decrement (D) stimuli in blue-on-yellow selective S-cones stimulation. Representative averaged data from the group with hypothyroidism and the control group. Thin black line connects the peaks of the first negative wave of the Laplacian of the control group with the abscise line.

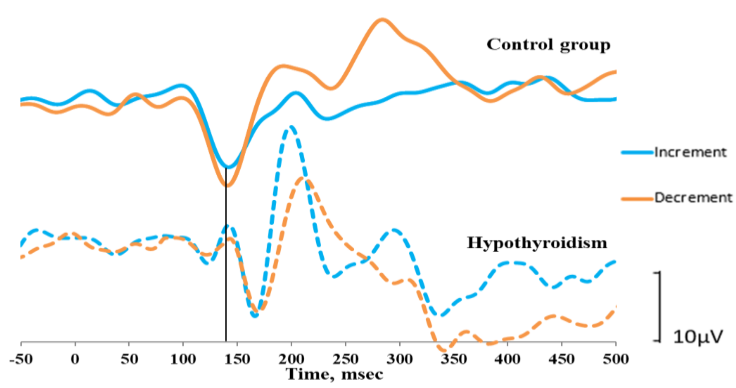


Figure 2 illustrates the Laplacians obtained in the conditions of selective S-cones stimulation in response to increment and decrement stimuli. It is seen that the signal-to-noise ratio is increased and the early negative wave of the Laplacians obtained exceeding the pre-stimulus fluctuations at least by a factor of three.
As Figure 2 shows the responses to the stimulus onset is characterised again by a negative-positive wave complex which appear between 136 and 154 msec for the control group and 16-30 msec later in the hypothyroidism. Indeed, the latency of the first negative wave was delayed in hypothyroidism and the difference was significant according to the t-test for both increment ($p<0.01$) as well as decrement ($p<0.01$) stimuli. The difference in the latency of the first negative wave which was noticeable in the monopolar VEPs (Figure 1), disappeared in the Laplacians and was not significant ($p=0.4$).
The bright yellow background which was used to adapt rods as well as L- and M- cones was causing discomfort for the observers. In order to avoid such effect, we perform the second experiment with a silent substitution method. We decided to present stimuli at 20° eccentricity because the results of our previous studies showed that at high eccentricity perceptual changes could be detected earlier (Vassilev et al., 2003; Racheva et al., 2019; Racheva et al., 2019b).
Figure 3 shows representative VEPs recorded from position Oz in response to isoluminant “blue” stimulus. The three VEPs are recorded in different contrast – 2.5, 4 and 8 above the detection threshold. The increasing of the contrast level leads to enlargement of the amplitude of the first negative wave and shortening of its latency.

Figure 3. VEPs, registered in response to a “blue” stimulus located at 20° eccentricity. The right labels show which line corresponds to which contrast. Data of one subject with hypothyroidism.

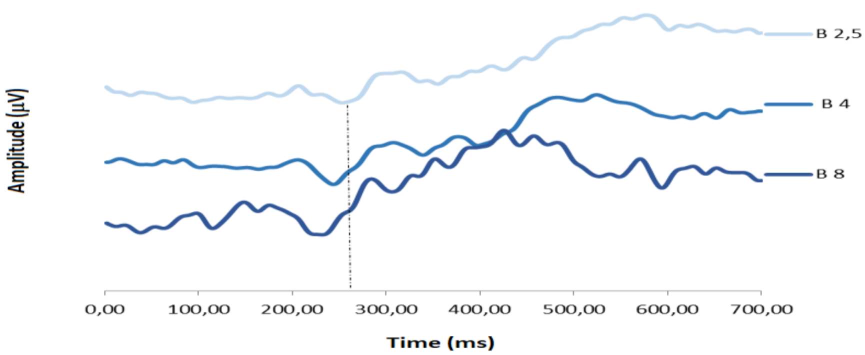
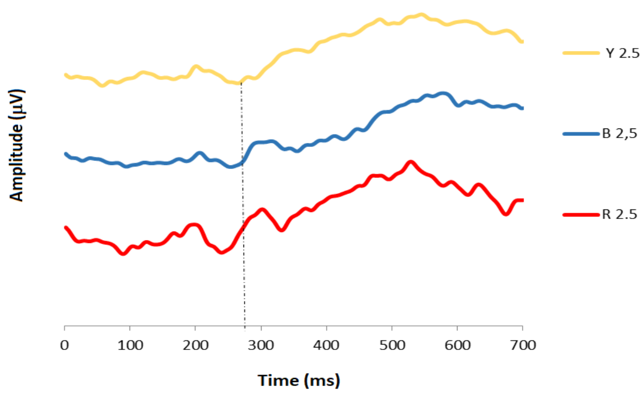


Figure 4 illustrates the comparison of the VEPs recorded at the equal above threshold contrast at different colour – “yellow”, “blue” and “red”. It is seen that the VEP responses to the “yellow” and “blue” stimuli are with much smaller amplitude and longer latency of their first negative wave compared to the VEPs to the “red” stimulus.
It turned out that it is most difficult to record VEPs in response to green stimuli because of limitations of spectra of the stimulation monitor as shown in other studies (Zlatkova et al., 2021).
Although the good quality of the VEP recoded in isoluminant stimulation in this observer, we could not register VEPs with enough signal-to-noise ratio in all other participants from both groups – with hypothyroidism as well as the control group.

Figure 4. VEPs registered in response to stimuli with a diameter of 4° and located at 20° eccentricity for the three colours shown on the right. Data of one subject with hypothyroidism.



4. DISCUSSIONS

The results obtained in the present study show that it is possible to record VEPs in response to selective S-cones stimulation in hypothyroidism by means of both approaches, the modified method of Stiles as well as the isoluminant stimulation. Many factors could cause the difficulties with recording VEPs in response to the isoluminant stimuli with some of the observers. One of them is the eccentric stimulus location and potential difficulties to retain stable fixation for a long time. Any eye movements could influence the VEP recording and diminish its quality introducing high amplitude noise. Another problem could be connected to the individual specifics in the morphology of the primary visual cortex, V1, and the location of the electrical dipoles in respect to the electrodes. However, the good quality of the VEPs recorded in our patient with hypothyroidism as well as the differences in the amplitude and the latency of the first negative VEP wave depending on the stimulus colour looks promising for further investigations. Probably, it is necessary to present stimuli with larger diameter or at lesser eccentricity in order to improve the quality of the sweeps recorded. As concerns to the improvement of the blue-on-yellow method, the only possibility to minimize the subjective discomfort is to shorten the stimulation time and to separate the experiment in different sessions with enough resting time between them.

The monopolar VEPs registered from the all three positions, PO7, Oz, PO8, showed larger amplitude and longer latency of the first negative and the next positive waves in response to decrement stimuli compared to incremental stimuli. Noteworthy, this asymmetry disappeared in the Laplacian derivations. The Laplacian analysis not only increases the signal-to-noise ratio, but also by sensing the local curvature of the potential field, attenuates the contribution from remote generators (e.g. Mihaylova et al., 1999). It could be suggested therefore that the more distant generators beyond the V1 contributed to the larger amplitude and delayed latency to decrement stimulation in the monopolar recordings.

The main results in the present study, the finding of the prolonged latency in hypothyroidism, broaden the previous findings of a delay in visual information processing in response to achromatic stimulation (Jaiswal et al., 2016). We suppose however, that the hypothyroidism's effects on the visual system's function could be detected earlier in the S-cones pathway by means of the selective stimulation.

In the second experiment with the silent substitution method the longer latency of the first negative VEP wave for yellow and blue stimuli was observed in comparison to the red one. This result is in line with the data of Bessler et al. (2010) who found longer latency of the S-cone response compared to the M- and L-cones response during isoluminant stimulation and provide an evidence for successful selective stimulation in our experiments. Psychophysical studies in hypothyroidism describe longer response for the yellow and blue stimuli (Zlatkova et al., 2019) and other deficits found with blue and yellow stimuli (Cakir et al., 2015; Racheva et al., 2019b; Racheva et al., 2020). This could be a result from a dysfunction in the S-cone pathway at a retinal level or in the early stage of visual processing.

In conclusion, the results of the present study obtained in the blue-on-yellow stimulation showed prolonged visual information processing in hypothyroidism, reflected in delayed VEP latency in response to both blue increments as well as decrements. Although promising results with prolonged response to yellow and blue stimuli compared to the red one in the silent substitution method, it is necessary to improve the stimulation conditions by increasing stimulus diameter or slightly decreasing the eccentricity of its presentation in order to obtain VEPs with better signal-to-noise ratio.

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