A new electrophysiological non-invasive method to assess retinocortical conduction time in the rat through the simultaneous recording of electroretinogram and visual evoked potential

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Background

Visual evoked potential (VEP) is the bioelectrical signal generated by visual cortex in response to visual stimulation. VEP is defined by 3 main components: P1, N1 and P2[1]. On the other hand, electroretinogram (ERG) records responses of retinal cells to visual stimuli. A first component, a-wave, represents responses from photoreceptors, whereas a second component, b-wave, reflects activity of the inner retina, mainly bipolar cells[2]. VEP is generally recorded in rodents invasively through epidural electrodes, while ERG can be recorded both invasively (with subscleral electrodes[3]) and non-invasively (with epicorneal electrodes[4]). Simultaneous recording of VEP and ERG allows evaluation of conduction time from retina to visual cortex, enabling to distinguish pathologies affecting retinocortical projections from others damaging retinal tissues.

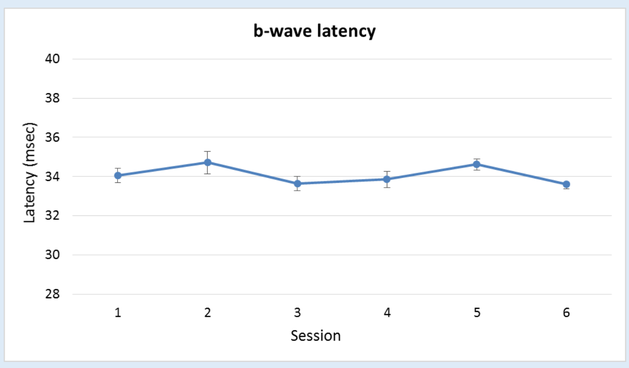
Aim of the Study

To develop a non-invasive method exploiting simultaneous recording of epidermal VEP and epicorneal ERG to study retinocortical function and to evaluate its reliability and repeatability over time.

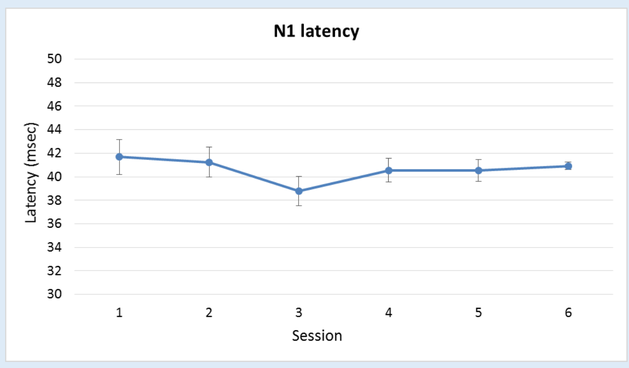
Materials and Methods

Female wild-type Dark Agouti rats (2 months of age) were anaesthetized with ketamine/xylazine (40/5 mg/kg). Epidermal VEP (Ag/AgCl cup electrode on scalp) and epicorneal ERG (gold ring electrode on eye surface) were recorded simultaneously in response to flash stimulation (3 trains of 20 stimuli of 10 µs duration and 1 Hz frequency) from both eyes of 5 rats (n = 10). One eye at a time was stimulated (with the other eye covered through a black silicon band). ERG was recorded from the stimulated eye, while VEP was recorded from the contralateral visual cortex. For VEP, cup active electrode was placed on the left or right visual cortex (+3.5 mm anterior to interaural line, ±4 mm lateral to midline). For both ERG and VEP, a needle electrode in the cheek was used as reference, while a needle in the hindlimb was used as ground. Body temperature was maintained at 37°C using a homeothermic heating pad. Pupils were dilated with tropicamide 1% and hydroxypropylmethylcellulose 2% was applied to prevent eye drying. Electrodes were connected through flexible cables to an amplifier (Micromed, Mogliano Veneto, Italy). Latencies and amplitudes of ERG b-wave and VEP P1, N1 and P2 were recorded. Retinocortical times were calculated as the difference between N1 and b-wave latencies (RCT1) and between P2 and b-wave latencies (RCT2). Coefficient of variation (Cov) across time was calculated as an index of repeatability: Cov% = 100 * (within standard deviation / within mean). Additionally, we computed a Cov adjusted for sample size, also known as relative standard error (RSE): RSE% = 100 * (within standard error / within mean).

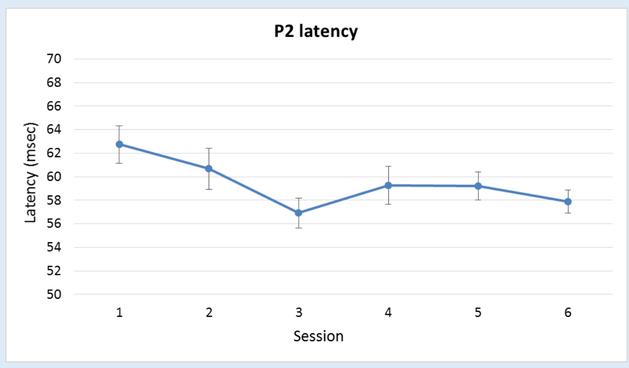
Results



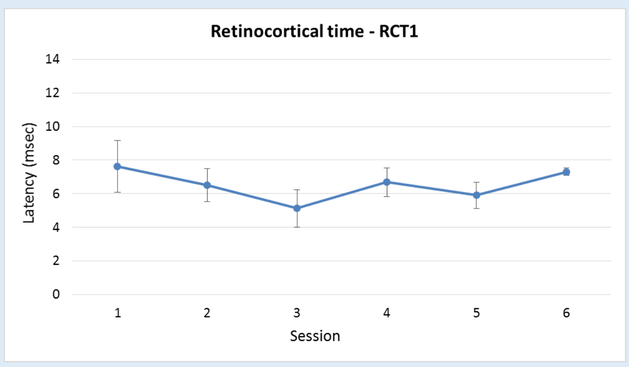
Latency of b-wave of ERG did not show any significant change over 6 weekly time points (One-way ANOVA: p = 0.172). Mean values ± SEM are represented.



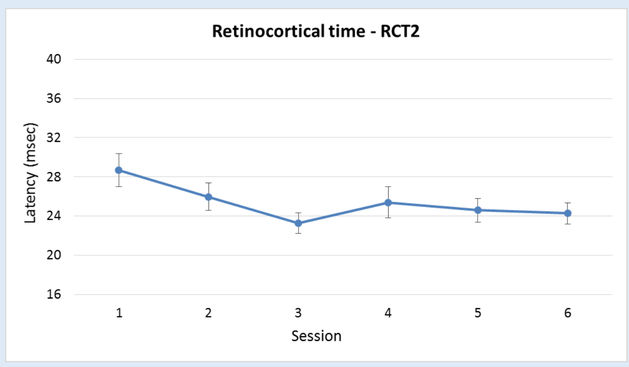
Latency of VEP N1 did not show significant changes over 6 weekly time points (One-way ANOVA: p = 0.457). Mean values ± SEM are represented.



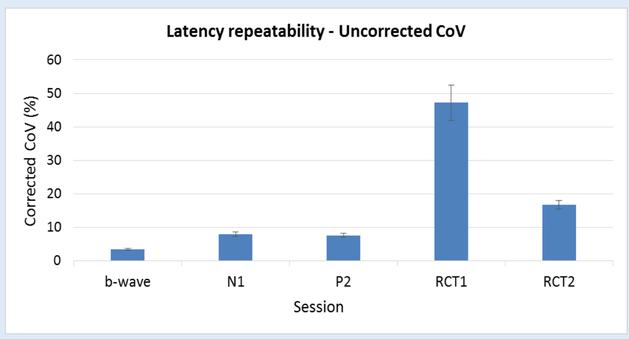
Latency of VEP P2 did not exhibit significant changes over 6 weekly time points (One-way ANOVA: p = 0.062). Mean values ± SEM are represented.



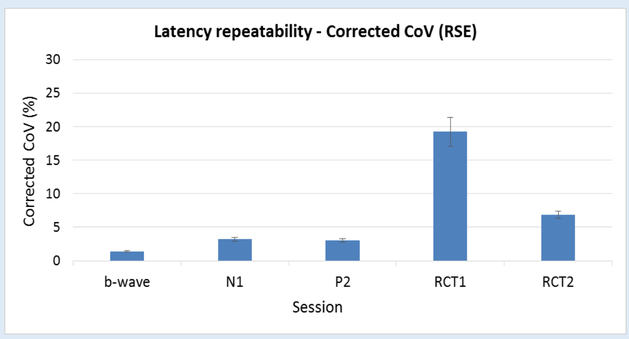
Retinocortical time from b-wave to N1 (RCT1) did not change over 6 weekly time points (One-way ANOVA: p = 0.426). Mean values ± SEM are represented.



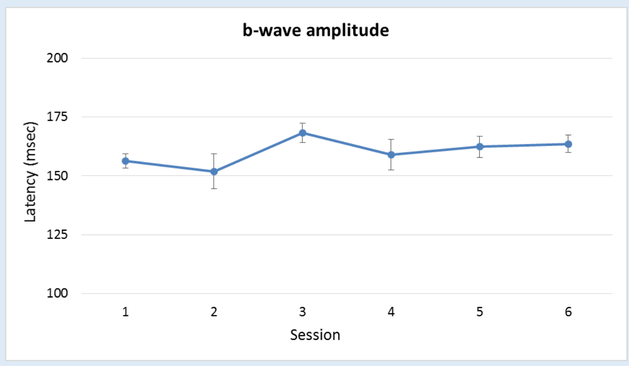
Retinocortical time from b-wave to P2 (RCT2) did not change over 6 weekly time points (One-way ANOVA: p = 0.086). Mean values ± SEM are represented.



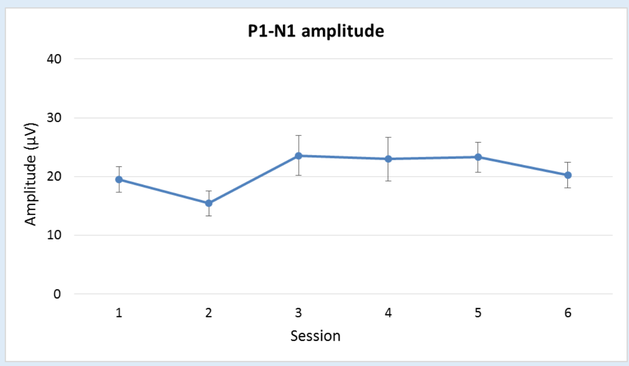
Latency repeatability analysis showed a good reliability over time for b-wave, N1 and P2 (Cov% <10%). Regarding retinocortical times, Cov% for RCT1 was <50%, while for RCT2 it was < 20%. Mean values ± SEM are represented.



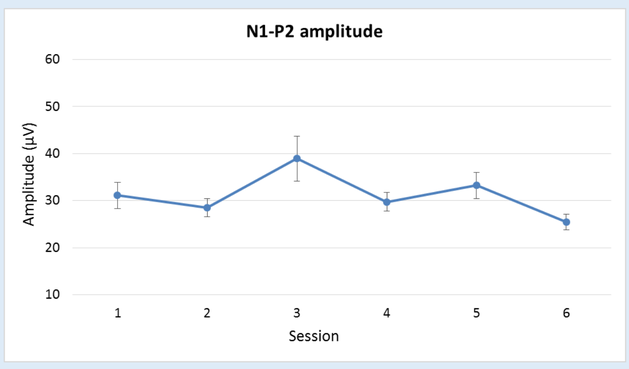
Corrected CoV% (RSE) confirmed a very good reliability over time for b-wave, N1 and P2 (<5%). Regarding retinocortical times, adjusted Cov% was acceptable for RCT1 (<20%) and good for RCT2 (<7%). Mean values ± SEM are represented.



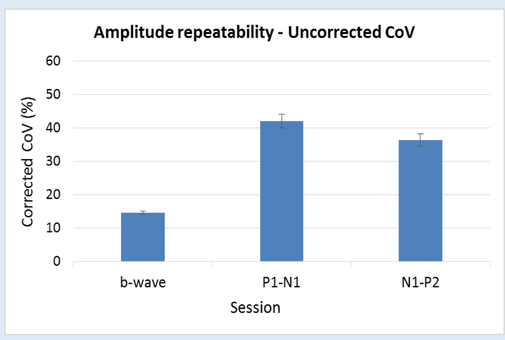
Amplitude of ERG b-wave did not show any significant change over 6 weekly time points (One-way ANOVA: p = 0.201). Mean values ± SEM are represented.



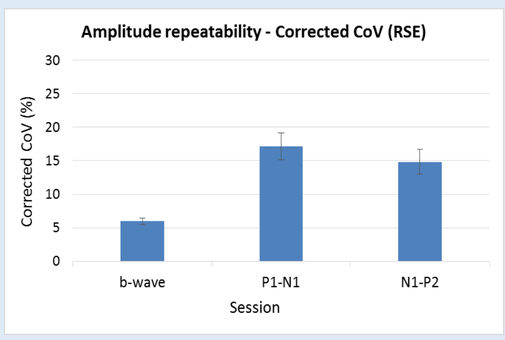
Amplitude of VEP P1-N1 did not present significant changes over 6 weekly time points (One-way ANOVA: p = 0.224). Mean values ± SEM are represented.



Amplitudes of VEP N1-P2 did not exhibit significant changes over 6 weekly time points (One-way ANOVA: p = 0.387). Mean values ± SEM are represented.



Amplitude repeatability analysis showed a good reliability over time for ERG b-wave (Cov% <15%). Regarding VEP, Cov% for P1-N1 amplitude was <45%, while for N1-P2 it was < 40%. Mean values ± SEM are represented.



Corrected CoV% (RSE) confirmed a good reliability over time for b-wave (Cov%~5%). Regarding VEP, Cov% for P1-N1 was <20%, whereas for N1-P2 it was < 15%. Mean values ± SEM are represented.

Conclusions

Simultaneous recording of ERG and VEP has been previously achieved through invasive methods requiring surgery[5]. Here we present a new non-invasive method, which allowed to obtain latencies and retinocortical times that were constant across a long period (6 weekly time points) and had a good CoV% over time (corrected CoV%: <5% for b-wave, N1 and P2 latencies; <20% and <7% for RCT1 and RCT2, respectively). ERG and VEP amplitudes were also stable over time, with good corrected Cov% (~5% for b-wave and ~15% for P1-N1 and N1-P2). Through the employment of removable electrodes, this method allows to assess retinocortical function in long follow-up studies. Moreover, its non-invasiveness reduces animal distress and avoids brain and eye lesions that could interfere with physiological responses of the visual system.

Bibliography

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