Stable Microsaccades and Microsaccade-Induced Global Alpha Band Phase Reset Across the Life Span

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Citation: Gao Y, Huber C, Sabel BA. Microsaccades are well preserved in aging and can be used as reference points for studying neurodegenerative or neuro-ophthalmologic diseases where the oculomotor system is affected. Microsaccade-induced alpha band activity is a potential biomarker to better understand and monitor these diseases, and we propose that microsaccades trigger "cortical refreshment" by resetting alpha band phase globally to prepare (sensitize) the brain for subsequent visual processing.

Keywords: microsaccade, spike potential, microsaccadic lambda response, alpha band synchronization, aging

Microsaccades are small, fast, and jerk-like eye movements that refresh vision by preventing photoreceptor adaptation and scan relative small and local visual fields. In addition, their corollary activities might also interact with ongoing brain oscillation to prepare visual centers for upcoming stimuli. Although one corollary activity is the microsaccade-induced gamma band response, there is a debate as to whether it represents electromyogenic artifacts or changes in cognitive processing. However, microsaccade-related brain activities are unlikely mere artifacts according to recent evidences that they serve as noninvasive electrophysiologic probes of attention and that they may modulate gamma activity to facilitate visual processing and information flow between brain regions.

This proposal is compatible with electrophysiologic studies showing two typical microsaccade-locked brain potentials: the spike potential (SP) and the microsaccadic lambda response (MLR). Whereas the SP is a summation of electromyography (EMG) spikes at microsaccade onset, the MLR signals occipital activation by microsaccade-induced retinal slip. Following the MLR peak, several cycles of alpha ringing at occipital sites were observed. However, this phenomenon cannot be fully captured by averaged event related potentials (ERPs), which probe temporal-spatial dynamics of brain activities. To overcome this limitation, we now applied time frequency analyses to characterize the microsaccade-related spectral power (event-related spectral perturbations [ERPS]) and phase (intertrial coherence [ITC]). ERPS measure the time course of relative changes in the spontaneous EEG amplitude spectrum induced by experimental events. ITC, a measure of cortical synchrony, on the other hand, reflects the temporal and spectral synchronization of brain oscillatory activity.

Clearly, microsaccades are not just important for normal visual processing but are also clinically relevant. In fact, microsaccades are altered in different ophthalmologic and neurologic diseases. For example, progressive supranuclear palsy patients display enlarged microsaccade amplitude and a loss of the vertical component; a disturbed microsaccade binocular conjugacy and direction bias were reported in hemianopia after stroke; altered microsaccade direction was observed in Alzheimer’s disease and mild cognitive impairment; and amblyopic eyes show fewer microsaccades and increased amplitudes, increased peak velocities, and longer intersaccadic intervals.

Because these diseases often occur in different age groups, we need to learn if microsaccade features are age dependent. Although age-matched normally sighted subjects are widely used for comparison with patients, reference values for the
different microsaccade features across age are still missing. Saccades, larger-type eye movements, are on the same continuum and share the same singular generator circuits with microsaccades. Involuntary saccades were found to be stable across different age groups, but if this is also true for microsaccades, which are also involuntary, is unknown.

To this end, we assessed microsaccades and their physiologic responses in subjects aged 18 to 77 years with the hypothesis that they remain stable across the life span as involuntary saccades do. In addition, we explored microsaccade-related potentials and temporal-spectral power and synchronization dynamics to learn more about their role in visual processing.

METHODS

Participants

For this cross-sectional study, we recruited young (n = 22, 18 to 29 years; mean ± SD, 23.8 ± 3.4 years; 13 female), middle-aged (n = 22; 31 to 55 years; 45.5 ± 7.9 years; 13 female), and elderly participants (n = 22; 56 to 77 years; 64.7 ± 5.7 years; 13 female). All subjects had normal or corrected to normal visual acuity (20/20) and no visual field deficits as checked perimetrically. Exclusion criteria were a history of hemianopia, amblyopia, glaucoma, cataract, diplopia, nystagmus, cataract, Parkinson’s disease, Alzheimer’s disease, and other disorders affecting the visual and oculomotor function. Written informed consent was obtained from all participants according to the Declaration of Helsinki (International Committee of Medical Journal Editors, 1991) after the institutional review board approved the study protocol.

Experimental Protocol

Participants were instructed to maintain fixation at a fixation dot on a gamma-corrected monitor (EIZO CG241W, resolution of 2560 × 1440; EIZO Corp., Ishikawa, Japan), which was placed at 67-cm distance. The white fixation dot (size: 10 pixels, luminance: 90 cd/m²) was presented against a gray background (luminance: 29 cd/m²) in 40 trials lasting 7 seconds each. The interstimulus interval was 3 seconds, during which participants could rest their eyes. All participants were tested individually in a silent, dimly lit room.

Eye Movement Recording

Binocular eye movements were recorded during the fixation task using an EyeLink-1000 system (SR Research, Ontario, Canada) with a sampling rate of 500 Hz and a spatial resolution of 0.01°. Head position was stabilized with a chin and forehead rest during eye tracker calibration, validation, and recording.

EEG Recording

During the fixation task, dense array EEG was simultaneously recorded with the eye movement data, using a HydroCell GSN 128-channel net and Net Amps 300 amplifier (EGI, Inc., Eugene, OR, USA). Our recording used a sampling rate of 500 Hz, a 200-Hz antialiasing low-pass filter, Cz as a reference (ground electrode between Cz and Pz), and was digitalized with 24-bit precision. Impedance was kept below 100 kΩ throughout the recording. Common trigger pulses were sent from the presentation computer to both eye tracking computer and EEG recording device for offline coregistration of eye movements and EEG.

Data Analysis

Continuous EEG signal was re-referenced to the linked mastoids and filtered with a high-pass (1 Hz) finite impulse response (FIR) filter, low-pass (100 Hz) FIR filter, and notch (50 Hz) FIR filter. Eye tracking data and EEG data were synchronized, and the synchronization quality was checked manually. Out-of-range eye tracking and EEG data were removed when there was a blink or when the eyes were not focused on the fixation point centered in a 4° × 4° window. Microsaccade detection was performed based on the algorithm proposed by Engbert et al. A microsaccade was defined by the following criteria: (1) the velocity exceeded six median-based SDs of the velocity distribution; (2) the duration exceeds 12 ms; and (3) the intersaccadic interval exceeded 50 ms, otherwise only the largest microsaccade would be kept. Then, binocular microsaccades were defined as those that occurred in left and right eyes with a temporal overlap. The criterion to confirm microsaccade detection validity was the main sequence relationship between microsaccade amplitude and microsaccade velocity. Microsaccade features include the following: rate (microsaccade number divided by detection time window length), amplitude (the Euclidean distance between the start and end point of the movement), velocity (the peak velocity during one microsaccade), duration, binocular microsaccade percentage (the proportion of binocular microsaccades in all detected microsaccades), and horizontal and vertical binocular disconjugacy indices.

Binocular microsaccades were added as “events” into the EEG data, and epochs locked to them (−0.5 to 0.5 seconds) were extracted, baseline corrected (−0.2 to 0 seconds), and manually screened for artifacts and noisy channels (on average, 70.1 ± 85.1 epochs per subject discarded; 7.4 ± 5.2 channels per subject discarded and interpolated based on the activity of surrounding channels). On average, 117.9 ± 76.3 epochs in the young group, 147.1 ± 62.2 epochs in the middle-aged group, and 137.9 ± 82.5 epochs in the elderly group were analyzed. We quantified two typical microsaccade-related potentials (i.e., MLR and SP). Potential latencies were defined in grand averaged ERPs for each of the three groups. Within a short window around it (20 ms for MLR; 10 ms for SP), individual peak potential amplitude and latency were located. The EEGlab function “newtimed” was used to compute ERSP and ITC across 25 linearly spaced frequencies ranging from 6 to 50 Hz and 200 linearly time points spanning −220 to 220 ms around microsaccade onset. Representative electrodes were selected for the occipital region (E75, E70, and E85), central region (E7, E31, E55, E80 and E106), and frontal region (E11, E19, and E4) (Fig. 1). We subdivided the time range into eight small time windows (−220 to −150, −150 to −100, −100 to −50, −50 to 0, 0 to 50, 50 to 100, 100 to 150, and 150 to 220 ms), and the frequency range into six bands (alpha: 7–14 Hz, low alpha: 7–11 Hz, high alpha: 11–14 Hz, beta: 14–30 Hz, low beta: 14–18 Hz, and high beta: 18–30 Hz). For source estimation, the forward model and the inverse model were calculated with an open access software Brainstorm. We calculated the forward model using the symmetric boundary element method (BEM) and default Montreal Neurological Institute (MNI) magnetic resonance imaging (MRI) template. The inverse model was estimated using the weighted minimum norm estimate (wMNE). When computing the inverse model, (1) the source orientations were constrained to be normal to the cortical surface; (2) a depth weighting algorithm was used to compensate for any bias affecting the superficial sources calculation; and (3) a regularization parameter, J = 0.1 was used to minimize numerical instability, reduce the sensitivity of the wMNE to noise, and effectively obtain a spatially smoothed
solution. In current density distribution visualization, a 1-Hz high-pass filter and 40-Hz low-pass filter were applied.

**Statistical Analysis**

ANOVA was used to test age effect on microsaccade features (binocular microsaccade percentage, microsaccade rate, amplitude, velocity, duration, and horizontal and vertical disconjugacy indices) and the amplitudes and latencies of SP and MLR. Before conducting between-group statistical tests, a normality of distribution was assessed with the Kolmogorov-Smirnov test of normality. For measures violating the normal distribution assumption, the Kruskal-Wallis test was used. To correct for multiple comparisons (nine comparisons conducted), a criterion of $P = 0.005$ (two-tailed) was set according to Bonferroni correction.

ERSP and ITC values within different time windows, frequency bands, and locations were averaged separately for each subject and analyzed in group $3 \times location \times frequency \times time$ repeated-measures ANOVA. Due to the violation of sphericity assumption, Greenhouse-Geisser adjustment was applied to the degrees of freedom in the repeated-measures ANOVA. Bonferroni correction was applied to all post hoc tests and simple effect tests.

**Software**

The experiment was developed in SR research Experiment Builder. Data analysis was conducted using Matlab 2016 and the following open access toolboxes: EEGlab and EYE-EEG extension (http://www2.hu-berlin.de/eyetracking.eeg, in the public domain). Statistical analyses were performed using IBM SPSS Statistics 23 (IBM; http://www.ibm.com/software/analytics/spss/, in the public domain).

**RESULTS**

The analysis of microsaccade features was carried out in all subjects, but EEG signals from 12 subjects had to be excluded from analysis due to lack of eligible epochs (less than 50 epochs) in the recording so that this analysis was carried out only in 19 young, 17 middle-aged, and 18 elderly subjects. To determine whether the excluded subjects had lower fixation performance or fewer microsaccades, we examined their eye tracking and EEG data and found that (1) their fixation performance did not differ significantly from the included subjects; (2) only one young subject showed lower microsaccade rate ($< 2 SD$), whereas the other subjects showed similar microsaccade rate compared with the included subjects; and (3) eight subjects (two young, four middle-aged, and two elderly) ended up with fewer epochs due to noise in EEG signal.

**Microsaccade Features and Microsaccade-Related Potentials**

Figure 2 displays the main sequences and angular distributions of microsaccades in three age groups. The main sequences for the three age groups were similar, and the microsaccade orientations exhibited a similar horizontal preference in three age groups, which is typical in normal subjects. Microsaccade and microsaccade-related potential features in the three age groups are shown in the Table. ANOVA revealed all microsaccade features to be comparable between the three groups, except that the elderly group showed higher microsaccade velocity compared with the young group ($P = 0.017$). However, this difference did not survive Bonferroni correction. Figure 3 illustrates the distribution of each microsaccade feature in three age...
groups with horizontal lines indicating 5% and 95% percentiles. Figure 4 shows the grand average ERPs of three age groups at occipital and central regions, the scalp distributions, and the current density maps of the SP and MLR. SP began around 5 ms before microsaccade onset and peaked around 16 ms after microsaccade onset. The scalp distribution of the SP displayed a positive deflection at central and occipital regions and negative deflection at the frontal region. The visualization of the source estimation result displayed a high current density in the anterior region close to the eyes. MLR began around 120 ms and peaked around 160 ms after microsaccade onset. The scalp distribution of the MLR exhibited a positive deflection occipitally and a negative deflection centrally. The visualization of the source estimation result displayed a high current density in the posterior part of the brain. ANOVA results

To correct for multiple comparisons, a criterion of $P = 0.005$ (two-tailed) was set for the ANOVAs according to Bonferroni correction.

*Kruskal-Wallis test was used, and $\chi^2$ values are reported due to violation of the normal distribution assumption. Degree of freedom was 2.
revealed no significant differences in the latencies and amplitudes of the SP and MLR between the three groups.

Microsaccade-Related Temporal-Spectral Power and Synchronization Dynamics

With respect to temporal-spectral power and synchronization dynamics, the overall group effect was not significant ($F[4,102] = 1.23, P = 0.304, \eta^2 = 0.05$). Figure 5 displays the ERSP and ITC change over time in different frequency bands over the occipital, central, and frontal regions. There was a significant interaction between electrode location, time, and frequency band in both ERSP and ITC ($F[11.4,583.7] = 4.41, P < 0.001, \eta^2 = 0.08; F[9.5,483.1] = 6.91, P < 0.001, \eta^2 = 0.12$). Simple effect analysis revealed the detailed temporal-spectral pattern after microsaccade onset: (1) alpha band ERSP increased and peaked within 100 to 150 ms in the occipital region (Figs. 5A, 5B) and ITC increased and peaked within 150 to 220 ms in all regions (Figs. 5C, 5D); (2) beta band displayed no significant ERSP change in any region over time (Figs. 5A, 5B); (3) low beta ITC increased and peaked within 150 to 220 ms in occipital and central regions, whereas in the frontal region, it peaked within 0 to 50 ms; and (4) high beta ITC increased and peaked within 0 to 50 ms in all regions (Figs. 5C, 5D) (all $P < 0.05$ after Bonferroni correction).

DISCUSSION

We report the first comprehensive assessment of microsaccade features, microsaccade-related potentials, and temporal-spectral power and synchronization dynamics in a group of 66 subjects of ages between 18 and 77 years. As we hypothesized, microsaccade features were found to remain stable across the life span. Furthermore, we uncovered microsaccade-induced occipital alpha band perturbation and enhanced global alpha band cortical synchrony within the MLR time window, which was also age independent.

Microsaccade-Related Temporal-Spectral Power and Synchronization Dynamics

Our temporal-spectral analysis captured characteristic oscillatory dynamic patterns after microsaccades in the alpha and beta frequency bands, and this dynamic pattern was maintained across the life span. Beta band spectral power was stable, but high beta ITC increased globally within the SP time window. The alpha band showed spectral perturbation locally.
in the occipital region and increased ITC globally within the MLR time window. These oscillatory dynamic patterns after microsaccades were observed during the time windows of both microsaccade-related potentials. We therefore need to consider two types of neuronal responses that contribute to ERP waveforms: the “evoked” type (phasic increase of postsynaptic activity in neuronal ensembles) and “phase reset” type (reorganization of the phase of ongoing neuronal oscillations).40 It has been suggested that an increased ITC that accompanies oscillatory power enhancement is not considered to be a “phase reset” response but an “evoked” response.40 To gain more insight into the mechanism underlying our finding of enhanced cortical synchrony, we compared the ERSP and ITC graphs (Fig. 5). Within the SP time window, globally increased high beta ITC was not accompanied by increased ERSP, suggesting a “phase reset” function, perhaps induced by EMG signals, which accompanies microsaccade execution. Within the MLR time window, enhanced alpha band cortical synchrony coincides with increased ERSP in the occipital region, suggesting the existence of an “evoked” response, whereas in the frontal and central regions, increased cortical synchrony existed without the presence of increased ERSP. This is an indication that “phase resetting” is taking place as well and is most visible outside the occipital region. Unlike intracortical electrode recording used in animal studies, which can probe neuron firing fluctuation and ongoing oscillation phase reset, with our EEG scalp recording, we are unable to determine in the occipital region whether the enhanced cortical synchrony is a purely “evoked” response or a combined “evoked” and “phase reset” response, although we think that the latter is more likely. New studies are now needed to better understand the mechanism underlying such alpha band synchronization.

While our results experimentally confirmed previous observations by others of alpha resynchronization following MLR peak,9 we now present for the first time the alpha band power and synchronization dynamics underlying this phenomenon. Our findings suggest that microsaccades induced not only spectral perturbation locally in the occipital region, but they also reset alpha band phase globally (i.e., in frontal, parietal, and occipital regions). We propose that such alpha band synchronization plays an important role in visual perception, and, in a speculative spirit, they might function to help synchronize visual processing with other functional systems, which might relate to the “binding” problem.41 For example, as one theory of microsaccade function indicates, saccade-related corollary activity plays a crucial role in anticipatory preparation of visual centers for visual processing.2 In fact, phase and amplitude of alpha activity are known to determine whether a stimuli reaches sensory awareness or not,42-45 and the saccade-induced phase is similar to the ongoing phase that leads to the maximal stimulus evoked response.7 In summary, we propose that microsaccades play a significant role in vision not only through retinal image refreshing,31 which counteracts visual fading, but also through “cortical refreshment” (i.e., resetting alpha band phase globally to prepare or sensitize the brain for subsequent visual processing). Future studies should further clarify the mecha-
Figure 5. ERSP and ITC changes over time are displayed in different frequency bands over the occipital, central, and frontal regions. (A) ERSP change across eight time windows in six frequency bands over the occipital, central, and frontal regions. (B) ERSP change over time across 7- to 30-Hz frequencies over the occipital, central, and frontal regions. (C) ITC change across eight time windows in six frequency bands over the occipital, central, and frontal regions. (D) ITC change over time across 7- to 30-Hz frequencies over the occipital, central, and frontal regions. In A and C, time scales are as follows: 1 = −220 to −150 ms; 2 = −150 to −100 ms; 3 = −100 to −50 ms; 4 = −50 to 0 ms; 5 = 0 to 50 ms; 6 = 50 to 100 ms; 7 = 100 to 150 ms; 8 = 150 to 220 ms. The vertical black lines represent microsaccade onset.
nimics of “cortical refreshment” to uncover their contribution in visual processing and its relationship with other non-visual functions in normal and abnormal vision.

Microsaccades Across the Life Span

All microsaccade features (binocular microsaccade percentage, microsaccade rate, amplitude, velocity, duration, and horizontal and vertical binocular disconjugacy) were comparable between the three age groups. This is compatible with what is known about involuntary saccades, which, unlike voluntary saccades, were relatively impervious to aging. This implies that, although voluntary saccade function declines with age, involuntary saccade function does not. As we now showed, this is true also for the involuntary microsaccades: subjects ranging in age from 18 to 77 years showed similar abilities to generate microsaccades and maintain binocular coordination.

Our results are not at odds with another seemingly contradictory report, which investigated microsaccade features during a puzzle solving task in subjects of different ages (4 to 66 years) where slightly increasing microsaccade rates were reported with increasing age. We did not observe such change, which might be due to the differences in the experimental tasks. We believe that the cognitive load of the puzzle task, which is sensitive to aging, might interact with microsaccade rate modulation. In contrast, we used a simple fixation task to avoid any cognitive load. In fact, it is known that elderly subjects perform worse in relatively difficult eye movement tasks requiring inhibition, high attention demand or wider attention span.

The slightly higher microsaccade velocity in our elderly group should be considered as preliminary evidence as it was not significant after Bonferroni correction. We briefly discuss it here as a reference for future studies. Studies about age effects on saccade velocity exhibited a discordant variety of findings. Whereas some studies revealed no age effect on saccade peak velocity, others discovered reduced velocity in the elderly population, especially in larger (>20°) saccades. Similar to our findings, some elderly subjects made faster saccades than the young ones, but the statistical results suggested no significant age effect on saccade velocity when conducting the group comparison. Future studies with larger samples of elderly subjects are needed to clarify this issue.

SP and MLR Across the Life Span

We also analyzed the typical microsaccade-related potentials across the life span. Figure 4 displays the averaged ERPs in the occipital and central regions, with time locked to microsaccade onset. SP is the spike peaking at about 16 ms after microsaccade onset, which has a positive deflection at central and occipital regions and negative deflection at the frontal region. It is the summation of the EMG signals accompanying microsaccade execution, which is further confirmed by its high current density near the eyes. MLR is the potential peaking at about 160 ms after microsaccade onset. It has positive polarity at the occipital and negative polarity at the vertex. At the MLR peak, high current density was found in the occipital lobe. MLR represents the occipital activation by microsaccade-induced retinal slip, which resembles the visually evoked potential (VEP). In the study of Dimigen et al., the MLR peaked at 100 ms after microsaccade onset with a mean amplitude of 9.6 μV. In our study, however, MLR peaked at 160 ms after microsaccade onset with a mean amplitude of 2.4 μV. This difference could be due to the different stimuli. Whereas we used a white dot presented against a gray background, Dimigen et al. used a black-and-white checkerboard. More intense visual stimuli can enlarge VEP amplitude and shorten their latency. We therefore believe that the less demanding stimuli used in our study resulted in smaller occipital activation caused by microsaccadic retinal slip, generating smaller MLR amplitude and longer MLR latency. Considering the age effect, we found that, like the behavioral microsaccade features, the amplitudes and latencies of the SP and MLR were stable across the life span.

To conclude, our most important findings are the microsaccade stability across age and the evidence for a new “cortical refreshment” function (i.e., resetting alpha band phase globally). This goes well beyond the understanding that microsaccades serve only the role of retinal refreshment. Our results have several implications: first, microsaccades are preserved relatively well throughout life. This stability is an excellent condition so that microsaccades can serve as reference points when studying age-associated neurodegenerative or neuro-ophthalmologic diseases with oculomotor symptoms; second, microsaccades’ function in vision is not limited to retinal refreshment, but they are also actively engaged in central visual processing, by possibly serving as a “pace maker” to cortical synchrony. This suggests the usefulness of microsaccades as a potential biomarker to monitor and better understand different diseases with oculomotor symptoms. In summary, microsaccades may be very small, but they are not too small to care when studying eye and brain function, dysfunction, and restoration.

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