

# Microsaccadic inhibition and P300 enhancement in a visual oddball task

MATTEO VALSECCHI,<sup>a</sup> OLAF DIMIGEN,<sup>b,c</sup> REINHOLD KLIEGL,<sup>b</sup> WERNER SOMMER,<sup>c</sup> AND MASSIMO TURATTO,<sup>a,d</sup>

<sup>a</sup>Department of Cognitive Sciences and Education, University of Trento, Rovereto, Italy

<sup>b</sup>Department of Psychology, University of Potsdam, Potsdam, Germany

<sup>c</sup>Department of Psychology, Humboldt-University at Berlin, Germany

<sup>d</sup>Center for Mind-Brain Sciences, University of Trento, Rovereto, Italy

## Abstract

It has recently been demonstrated that the presentation of visual oddballs induces a prolonged inhibition of microsaccades. The amplitude of the P300 component in event-related potentials (ERPs) has been shown to be sensitive to the category (target vs. nontarget) of the eliciting stimulus, its overall probability, and the preceding stimulus sequence. In the present study we further specify the functional underpinnings of the prolonged microsaccadic inhibition in the visual oddball task, showing that the stimulus category, the frequency of a stimulus, and the preceding stimulus sequence influence microsaccade rate. Furthermore, by co-recording ERPs and eye movements, we were able to demonstrate that, despite being largely sensitive to the same experimental manipulation, the amplitude of P300 and the microsaccadic inhibition predict each other only weakly.

**Descriptors:** Cognition, Normal volunteers, EEG/ERP, Oculomotor

During the last 10 years, the interest about eye movements during fixations has increased considerably. Due to the introduction of video-oculographic methods for the recording of eye movements, it is now possible to reliably identify microsaccades, which are fast (up to 300°/s) mainly conjugate eye movements occurring about once per second (Møller, Laursen, Tygesen, & Sjølie, 2002). Many relevant results have emerged. For example, it has been shown that microsaccades modulate the firing of neurons in the visual system (Leopold & Logothetis, 1998; Martinez-Conde, Macknik, & Hubel, 2000, 2002) by counteracting neural adaptation and the fading of peripherally presented stimuli during sustained fixation (Martinez-Conde, Macknik, Troncoso, & Dyar, 2006). This hypothesis is consistent with the finding that microsaccades become more frequent when the retinal displacement produced by slower fixational eye movements (i.e., drifts) is reduced (Engbert & Mergenthaler 2006). Microsaccades have also been found to play a role in the maintenance of correct visual fixation (Engbert & Kliegl, 2004; Liang et al., 2005; Mergenthaler & Engbert, 2007). Finally, there is growing evidence that the orienting of spatial attention biases the direction of microsaccades (Betta, Galfano, & Turatto, 2007; Engbert & Kliegl, 2003; Galfano, Betta, & Turatto, 2004; Laubrock, Engbert, & Kliegl, 2005; Laubrock, Engbert, Rolfs, & Kliegl, 2007; Rolfs, Engbert, & Kliegl, 2004, 2005; Turatto, Valsecchi, Tamè, & Betta, 2007; but see Horowitz, Fine, Fencsik, Yurgenson, &

Wolfe, 2007). Similar results have recently been confirmed on other high-speed fixational eye movements known as saccadic intrusions (Gowen, Abadi, Poliakoff, Hansen, & Miall, 2007).

Microsaccades also seem to be influenced by higher-level cognitive factors other than spatial attention. In his seminal work on fixational eye movements, Barlow (1952) described a reduction in the rate of microsaccades when participants were required to perform a demanding cognitive task. This was based largely on nonsystematic observations of the participants' behavior. However, recent works have confirmed that microsaccades are inhibited when participants encounter rare task-relevant visual stimuli, which have to be counted. Valsecchi, Betta, and Turatto (2007) measured the rate of microsaccades in a visual oddball task, which consisted of the serial presentation of rare target stimuli (oddballs) and frequent nontarget stimuli in random order. The authors found that the probability of microsaccades following the presentation of standard stimuli showed a biphasic time course, with an early inhibition phase peaking at 100–150 ms after stimulus onset and a later rebound phase peaking at 300–350 ms after stimulus onset. This stereotypical response has been widely observed in response to visual (Engbert & Kliegl, 2003; Galfano et al., 2004) and acoustic (Rolfs et al., 2005) stimuli, and is considered a subcortical oculomotor reflex possibly occurring at the superior colliculus level, at least in its inhibitory component (Engbert, 2006). However, Valsecchi et al. (2007) found that the inhibitory phase of the microsaccadic response was prolonged and the rebound almost abolished after the presentation of an oddball stimulus. This effect was observed both with peripheral and central stimuli and for different stim-

Address reprint requests to: Matteo Valsecchi, Department of Cognitive Sciences and Education, Corso Bettini 31, 38068 Rovereto (TN), Italy. E-mail: matteo.valsecchi@unitn.it

ulus onset asynchronies, but it was absent when the oddballs were not task relevant. The authors suggested that the prolonged inhibition of microsaccades could be considered an index of the evaluation of task-relevant stimuli in the visual oddball paradigm. In a more recent study (Valsecchi & Turatto, 2007), the authors again found the prolonged inhibition of microsaccades in response to visual oddballs, while also showing, by using stimuli equiluminant with the background, that a cortical visual pathway can support the modulation of microsaccade rate in response to both oddball and standard stimuli. Recently, Valsecchi and Turatto (2009) showed that oddball stimuli induce a prolonged inhibition of microsaccades also in the auditory modality, a finding consistent with the hypothesis that this effect is an index of later and nonmodality-specific stages of stimulus processing.

Oddball paradigms have been extensively used in psychophysiological studies for four decades (e.g., Donchin & Coles, 1988; Johnson, 1988; Näätänen, Gaillard, & Mäntysalo, 1978). A specific response to oddball or infrequent stimuli has been observed in several peripheral measures, such as galvanic skin response (e.g., Bahramali et al., 1997; Lim et al., 1999), heart rate (e.g., Lyytinen, Blomberg, & Näätänen, 1992; Simons, Graham, Miles, & Balaban, 1998), and pupillary dilation response (Friedman, Hakerem, Sutton, & Fleiss, 1973).

A large amount of evidence has been collected with respect to the P300 component in the event-related brain potential (ERP). The P300 is a centro-parietal positivity peaking at around 300 ms after stimulus onset. This component has been considered an index of stimulus categorization (Donchin & Coles, 1988; Kok, 2001; Kutas, McCarthy, & Donchin, 1977; Verleger, 1988) and has been shown to be sensitive to both the stimulus category, that is, targets induce a higher-amplitude P300 than nontargets, and its overall, a priori frequency, that is, the less frequent a stimulus, the larger the elicited P300 (e.g., Duncan-Johnson & Donchin, 1977). P300 amplitude is also modulated by stimulus sequence (Jentzsch & Sommer, 2001; Squires, Wickens, Squires, & Donchin, 1976), with disruptions of runs of stimulus repetitions or alternations eliciting larger P300 amplitudes than continuations of such runs. The sequence-based enhancement of P300 amplitude can be dissociated from the effect of overall stimulus frequency (Duncan-Johnson & Donchin, 1977).

Hence, microsaccadic inhibition and P300 enhancement are both observed in response to rare targets in visual oddball paradigms. However, it remains to be shown whether microsaccadic inhibition is also sensitive to target effects and stimulus sequence as is P300 amplitude. To answer these questions and to establish whether the similarity between the two measures goes beyond the sensitivity to the same experimental manipulations, we conducted a visual oddball experiment orthogonally manipulating stimulus frequency and stimulus category while simultaneously recording eye movements and ERPs. If a functional relationship exists between microsaccadic inhibition and P300 enhancement, we expected to find effects of stimulus category, stimulus frequency, and stimulus sequence on both measures. Moreover, if the two phenomena are directly related they could predict each other.

## Methods

### Participants

Thirteen young adults took part in the experiment. One participant's data were discarded from analysis because of the presence of blinks in more than 50% of the epochs. The mean age of the remaining 12 participants was 25.6 years; 9 were women. All

participants reported normal visual acuity, showed normal color vision according to the Ishihara Color Vision Test (Ishihara, 2003), and were right-handed according to the Edinburgh Inventory (minimum score = 64; Oldfield, 1971). Two of the authors (M.V. and O.D.) took part in the experiment, whereas all other participants were naïve as to the purpose of the study. The inclusion of two nonnaïve participants in the sample is potentially a confound, but its impact should be limited given that little voluntary control is expected on microsaccadic frequency and P300. All participants gave informed consent and were remunerated either with 7€ per hour or course credits.

### Stimuli

Stimuli were red or green disks (2.04° in diameter), with a white fixation dot (0.48° in diameter) at the center. To enhance the physical similarity between target and nontarget stimuli and to control for intensity effects, the luminance of the red and green colors was matched for each participant using 25-Hz flicker fusion (Ives, 1912). The background was black during the entire experiment. Stimuli were presented on a 19-in. LG Flatron 915FT CRT monitor at a refresh rate of 100 Hz and a viewing distance of 75 cm. Stimulus duration was 100 ms, interstimulus interval was 900 ms, and the fixation point remained visible during the interstimulus interval. Stimulus presentation was controlled using Presentation software (Neurobehavioral Systems, Inc., San Francisco, CA).

### Experimental Procedure

Participants sat in a dimly illuminated, acoustically and electrically shielded cabin. The experiment was divided into three conditions. In each condition, 500 stimuli were presented in random order. Participants had to silently count the stimuli matching the target color, which alternated between participants, and had to fixate the white dot while minimizing eyeblinks during the experimental sessions. After each block of 100 stimuli, participants reported the number of stimuli and were allowed to rest. Forty additional stimuli were presented in a practice block before each experimental condition. In the first condition, 50% of the stimuli were targets, whereas in the following two conditions the frequency of targets could be either 20% or 80% (the order of the last two conditions was alternated across participants). The 50% condition was always run first in order to avoid possible carry-over effects, that is, participants might otherwise have implicitly adopted a biased expectation about the global stimulus frequency from the previous condition.

### Eye-Movement Recording and Microsaccade Detection

Eye movements were recorded monocularly, with an iView X Hi-Speed infrared eyetracker (SensoMotoric Instruments, Teltow, Germany). Movements of the head were limited by the eye tracker's built-in chin and forehead rest. Recording was from the right eye, though viewing was binocular. The system had a sampling frequency of 238 Hz, a tracking resolution of <90 s-arc and an absolute gaze position accuracy of up to 0.2°. A standard 9-point calibration was performed before the beginning of each block of 100 stimuli. Fixation was checked after every 10 trials. If the gaze was found outside of a 2.04° × 2.04° square centered around the fixation point, the experiment was interrupted and the system recalibrated.

Microsaccades were detected using the algorithm introduced by Engbert and Kliegl (2003). The algorithm was applied to epochs ranging from 150 ms before stimulus presentation to 1050

ms after stimulus presentation. Microsaccades were defined as parts of the eye position trace where velocity (calculated with a 5-point moving window) exceeded a combined threshold for the vertical and horizontal component equal to six times the standard deviation of the velocity profile within the epoch. Minimum allowed duration was four samples (16.8 ms) and maximum allowed peak velocity was  $200^\circ/\text{s}$ . Additionally, microsaccades starting less than four samples after the previous microsaccade were rejected. Epochs containing blinks or saccades with amplitudes greater than  $1^\circ$  were discarded from analyses. Microsaccades were included in the analysis regardless of their spatial orientation.

### Electrophysiological Recording

The electroencephalogram (EEG) was recorded from 40 Ag/AgCl electrodes on the scalp and around the eyes. Thirty-four of the electrodes were mounted in an elastic electrode cap (Electrocap International Inc., Eaton, OH) at positions Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, FT9, FC5, FC1, FC2, FC6, FT10, T7, C3, Cz, C4, T8, CP5, CP1, CP2, CP6, P7, P3, Pz, P4, P8, PO9, O1, Oz, O2, PO10, and Iz (American Electroencephalographic Society, 1994). Foam cushions were fitted to the participant's forehead to avoid direct pressure on the frontal electrodes. Six external electro-oculogram (EOG) electrodes were affixed at the outer canthi of the left and right eyes, below each eye, and on the left and right mastoids. An electrode at AFz was used as ground. All impedances were kept below 5 k $\Omega$ . A Brainamp DC amplifier (Brain Products GmbH, Munich, Germany) digitized the data at a sampling rate of 250 Hz, and a bandpass from DC to 70 Hz. Data was recorded with a PC running BrainVision Recorder Software (Brain Products GmbH). All channels were initially referenced to the left mastoid (A1) and converted to average reference off-line. Synchronization between EEG and eye tracker was achieved via TTL pulses sent from the stimulus presentation PC to both systems on every trial. The co-registration setup used in the present study has previously been applied and evaluated in several psycholinguistic experiments on reading (Dimigen et al., 2006).

### Data Analysis

The eye tracking data and the ERP data were first analyzed separately. In particular, we extracted three measures of microsaccadic inhibition, that is, amplitude of the peak microsaccade rate, latency of the peak microsaccade rate, and rate of microsaccades in a specific time window of interest (WOI), and one measure of P300 amplitude, that is, the average voltage at electrodes P3, Pz, and P4 between 200 and 500 ms. Repeated-measures analyses of variance (ANOVAs) with Stimulus Category (target vs. nontarget) and Stimulus Frequency (20%, 50%, or 80%) as factors were performed on each of the different measures.

Subsequently, we looked for sequence effects on the microsaccade rate in the time WOI and on P300 amplitude. We identified continued and discontinued sequences of stimulus repetitions, which are known to generate different P300 amplitudes, in the 50% stimulus frequency condition, and we performed repeated-measures ANOVAs with Stimulus Category and Sequence as factors.

Finally, to explore the relationship between microsaccades and P300, within the 50% frequency target trials, we investigated whether the presence of a microsaccade in the time WOI and the P300 amplitude within a trial were predictive of each other.

Greenhouse–Geisser corrected degrees of freedom and p values are reported where applicable.

## Results

### Counting Task

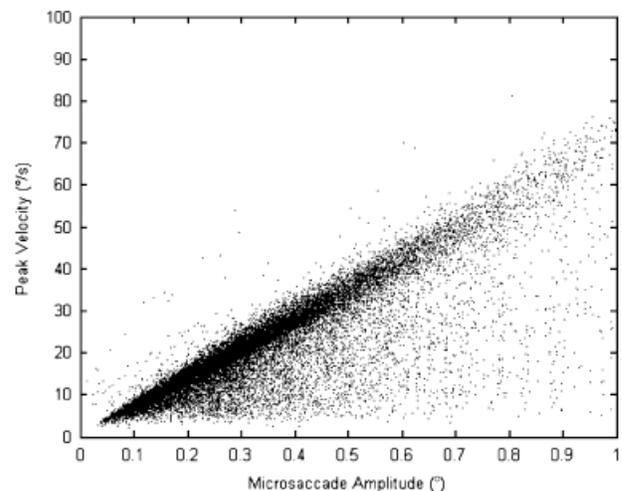
The participants were highly accurate in counting the target stimuli. The mean absolute counting error was 1.19% in the experimental condition with 50% targets, 0.91% in the condition with 20% targets, and 0.95% in the condition with a target frequency of 80%. No counting errors occurred in 77.09% of the reports. The counting data were not statistically analyzed.

### Microsaccade Rate: Stimulus Category and Frequency Effects

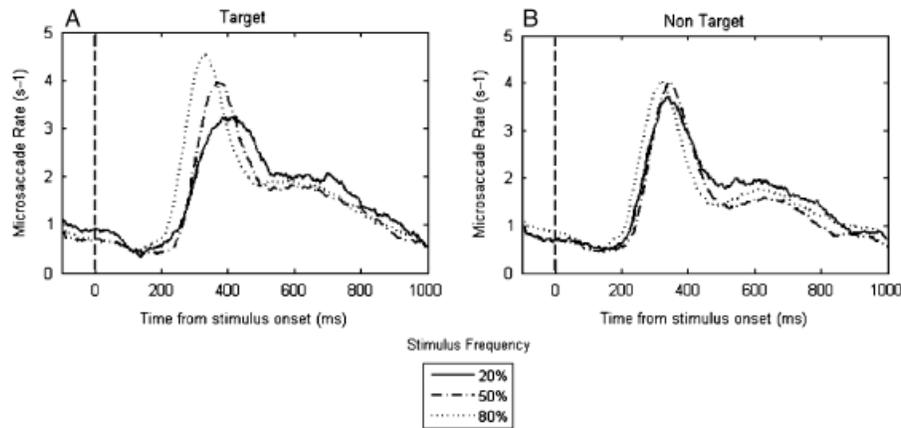
The minimum number of epochs for each cell of the experimental design (i.e., for each combination of participant, stimulus frequency, and stimulus category) was 36. The relationship between peak velocity and microsaccade amplitude, defined as the maximum displacement between any two points along the movement trace, is depicted in Figure 1. The linear relationship (main sequence) is indicative of the fact that the events detected by the algorithm are microsaccades (Zuber, Stark, & Cook, 1965).

The evolution of microsaccade rate in response to target and nontarget stimuli is depicted in Figure 2, separately for the three stimulus frequency levels (20%, 50%, and 80%). The rate was calculated in a sliding time window of 100 ms, moving in steps of 4.2 ms (i.e., the maximum temporal resolution allowed by the sampling frequency of the eye tracker). The plots were constructed for each participant, stimulus frequency, and stimulus category, and subsequently averaged across participants.

To ensure that the visual stimuli induced a reliable inhibition of microsaccades, we first identified the time point at which the minimum microsaccade rate was reached for each stimulus category and stimulus frequency. The average latency of the inhibition peak across stimulus category and stimulus frequency was 138.7 ms. The microsaccadic rates in two 100-ms bins, the first one centered on 0 ms latency (i.e., stimulus onset) and the second one centered on 138.7 ms latency (i.e., the inhibition peak), were analyzed in a three-way ANOVA with Bin (0 vs. 138.66 ms),



**Figure 1.** Microsaccade peak velocity as a function of microsaccade amplitude (defined as the maximum displacement between any two points along the movement trace). A clear linear relationship (main sequence) is evident.



**Figure 2.** Evolution of microsaccade rate in response to target (A) and nontarget (B) stimuli for the three levels of stimulus frequency (20%, 50%, and 80%). The rate has been calculated in a 100-ms-wide time window moving in 4.2-ms steps.

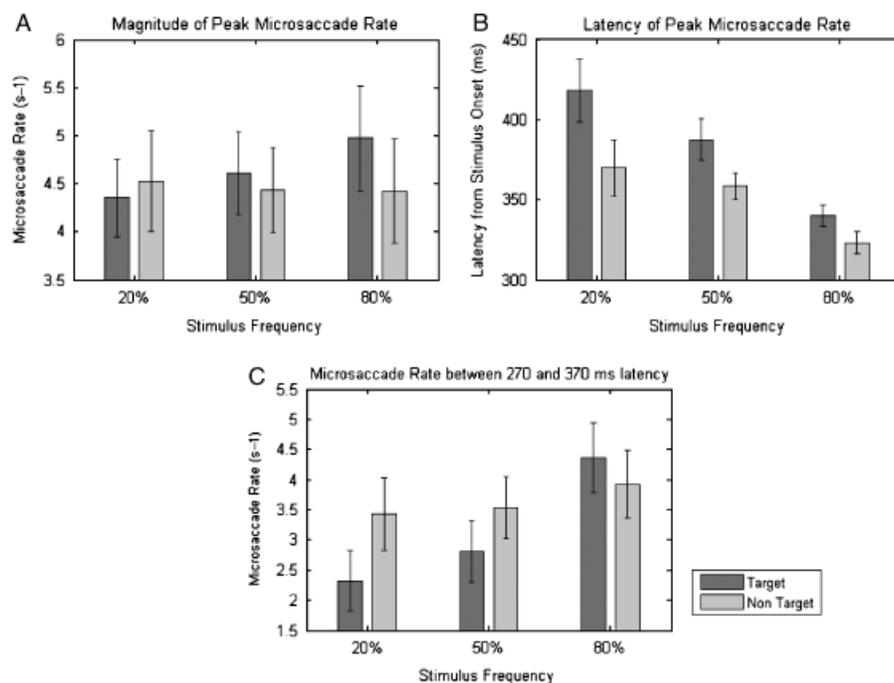
Stimulus Category, (Target vs. Nontarget), and Stimulus Frequency (20%, 50% or 80%) as factors. The main effect of Bin was significant,  $F(1,11) = 12.05$ ,  $p < .006$ , showing the overall presence of an inhibition effect. The Bin  $\times$  Stimulus Category and the Bin  $\times$  Stimulus Frequency interactions were not significant (both  $ps > .05$ ).

Of central interest for the present study, however, was the later rebound in microsaccade rate. A peak in microsaccade rate was reached in all conditions between 300 and 500 ms after stimulus onset. The magnitude of the peak rate and its latency seemed to be modulated by stimulus frequency and stimulus category, and this modulation was more evident in the case of target stimuli. In particular, with increasing target frequency, the amplitude of the peak microsaccade rate seemed to increase, whereas the latency of the peak seemed to decrease.

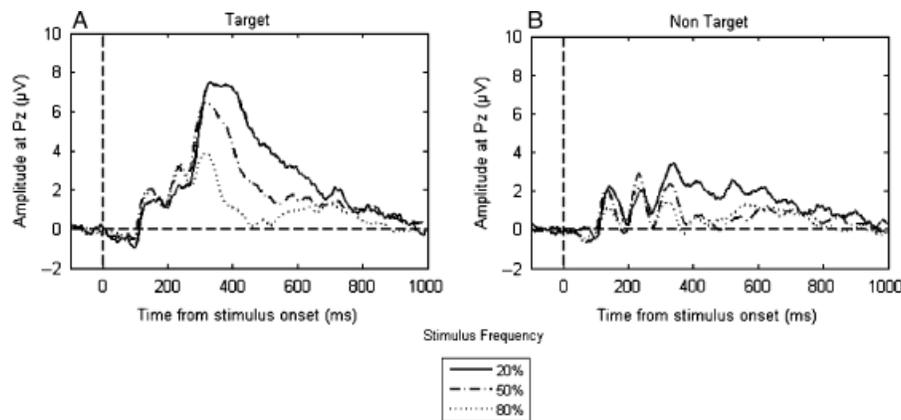
This observation was confirmed by a statistical analysis. The magnitude of the peak microsaccade rate and its latency could

be identified for each participant as the earliest point where the maximum value in microsaccade rate was reached in the single participant equivalent of the plots in Figure 2. Additionally, we calculated the microsaccade rate in a 100-ms time WOI centered on the latency of the peak microsaccade rate observed in response to the most frequent nontarget stimuli. The center of the WOI was set at 320 ms after stimulus onset according to the grand averages in Figure 2B (also see Valsecchi et al., 2007). A separate  $3 \times 2$  ANOVA with Stimulus Frequency (20%, 50%, or 80%) and Stimulus Category (target vs. nontarget) as factors was applied to each of the three measures.

In the case of the magnitude of peak microsaccade rate (Figure 3A), we did not observe a significant effect of Stimulus Category and Stimulus Frequency (both  $F_s < 1$ ), but their interaction was significant,  $F(1.59, 17.55) = 3.95$ ,  $p < .046$ . Post hoc tests were performed separately for the two stimulus categories. In the case of target stimuli there was a nonsignificant trend for



**Figure 3.** Mean magnitude of peak microsaccade rate (A), mean latency of peak microsaccade rate (B), and mean microsaccade rate in the time WOI between 270 and 370 ms after stimulus onset (C). Error bars are between-participant standard errors of the mean.



**Figure 4.** Evolution of ERP amplitude at Pz in response to target (A) and nontarget (B) stimuli for the three levels of stimulus frequency (20%, 50%, and 80%).

the magnitude of peak microsaccade rate to increase as a function of the stimulus frequency,  $F(1.46,16.04) = 3.05$ ,  $p < .088$ , whereas the peak microsaccade rate was unaffected by stimulus frequency in the case of nontargets,  $F(1.55,17.05) < 1$ .

The same analysis was applied to the latency of the peak microsaccade rate (Figure 3B) revealing significant effects of Stimulus Category,  $F(1,11) = 33.87$ ,  $p < .001$ , and Stimulus Frequency,  $F(1.26,13.95) = 12.10$ ,  $p < .002$ , whereas their interaction was only marginally significant,  $F(1.55,17.07) = 3.14$ ,  $p = .079$ . The latency of the peak microsaccade rate was shorter for nontargets and decreased as the stimulus frequency increased. At least numerically, the effect of stimulus frequency seemed to be stronger in the case of targets as compared to nontargets.

Stimulus Frequency had a significant effect also on the microsaccade rate in the time WOI,  $F(1.57,17.34) = 10.37$ ,  $p < .002$  (Figure 3C), whereas the effect of Stimulus Category was not significant,  $F(1,11) = 2.62$ ,  $p = .134$ . However, the interaction between the two factors was significant,  $F(1.74,19.19) = 21.62$ ,  $p < .001$ . Post hoc tests were performed separately for the two stimulus categories. The rate of microsaccades in the time window centered at 320 ms after stimulus onset increased as a function of Stimulus Frequency in the case of target stimuli,  $F(1.54,17) = 21.20$ ,  $p < .001$ , whereas the effect was not significant in the case of nontargets,  $F(1.69,18.55) = 1.49$ ,  $p = .249$ .

This interaction of Stimulus Frequency and Stimulus Category shows that rare stimuli compared to frequent stimuli elicited a stronger microsaccadic inhibition in the WOI only if they were targets, that is, if they needed to be counted.

### **P300: Stimulus Category and Frequency Effects**

EEG data were segmented into epochs extending from 100 ms before stimulus onset to 1000 ms after stimulus onset and baseline corrected by subtracting for each channel the mean voltage in the 100-ms prestimulus interval. ERP analysis was conducted only on epochs that had not already been discarded from the microsaccadic analysis, that is, where neither blinks nor saccades longer than  $1^\circ$  were detected. Furthermore, we discarded epochs where drift artifacts (defined as absolute voltage values in the epoch exceeding  $100 \mu\text{V}$  after baseline correction or a voltage difference between any two sampling points in the channel greater than  $150 \mu\text{V}$ ) were detected. The minimum number of epochs for each cell of the experimental design (i.e., for each combination of participant, stimulus frequency, and stimulus category) was 35. The grand average of the voltage amplitude

at electrode Pz in response to target and nontarget stimuli is depicted in Figure 4, separately for the three stimulus frequency levels (20%, 50%, and 80%). As in the case of microsaccade rate, there was a clear modulation of the waveform by stimulus frequency, and this seemed to be particularly evident in the case of target stimuli.

We chose to use the average voltage at electrodes P3, Pz, and P4 between 200 and 500 ms latency as a measure of P300 amplitude for statistical analysis. This choice was corroborated by the observation in the scalp topographic maps (not presented) that in this time window the experimental manipulations most strongly affected the voltage at centroparietal electrodes, which is typical of this paradigm.

Moreover, a control analysis showed that voltage at these electrodes is unlikely to be contaminated by corneoretinal artifacts (e.g., Gratton, 1998) due to microsaccades. To estimate the corneoretinal artifact introduced by microsaccades, we classified them as either leftward or rightward oriented according to the horizontal movement component, because the majority of microsaccades are horizontally oriented. A segment of EEG was then cut around the onset of each microsaccade in the oddball experiment and baseline corrected with a 100-ms premicrosaccade baseline. The mean voltage at the horizontal EOG electrodes (in a 100-ms interval following microsaccade onset) was used as an estimate for the corneoretinal artifact generated by microsaccades. The grand mean difference between the two electrodes (i.e., the bipolar-referenced EOG, left minus right electrode) in this interval was  $1.74 \mu\text{V}$  for leftward-oriented microsaccades and  $-2.08 \mu\text{V}$  for rightward-oriented ones. The fraction of artifact that propagates from the horizontal EOG to centroparietal electrode Pz has been estimated by Picton et al. (2000; see their Table 1) as 0.028 and 0.024 for leftward and rightward horizontal saccades, respectively. Therefore, a maximum possible distortion of around  $\pm 0.05 \mu\text{V}$  was expected at Pz. Furthermore, the distribution of microsaccadic direction in our experiment was quite symmetrical; around 50.1% of the microsaccades were rightward oriented. The actual distortion was therefore even smaller, due to the cancellation of artifacts from leftward- and rightward-oriented microsaccades in the averaging process.

A two-way ANOVA with Stimulus Frequency and Stimulus Category as factors revealed significant main effects of Stimulus Category,  $F(1,11) = 61.94$ ,  $p < .001$ , and Stimulus Frequency,  $F(1.58,17.48) = 29.14$ ,  $p < .001$ , on P300 amplitude. The two

factors also interacted significantly,  $F(1.36, 14.97) = 7.87$ ,  $p < .009$ . The effect of Stimulus Frequency was more pronounced for target than for nontarget stimuli. Nonetheless, post hoc tests indicated that the frequency effect was significant for both targets,  $F(1.38, 15.18) = 24.7$ ,  $p < .001$ , and nontargets,  $F(1.81, 19.91) = 7.51$ ,  $p < .005$ .

The pattern of ERP results was similar to the one we observed for microsaccades, with P300 amplitude affected by the interaction of stimulus frequency and category. The main difference between the two measures was that targets elicited a larger P300 amplitude compared to nontargets, whereas the target effect was not significant on microsaccadic rate. Notice that P300 amplitude decreased as a function of stimulus frequency, whereas the rate of microsaccades in the corresponding time window showed the opposite pattern, that is, it was higher for more frequent stimuli.

### Sequence Effects

Sequence effects on microsaccade rate and P300 amplitude were analyzed only in the case of the 50% stimulus frequency condition, where all sequences of a given order were equiprobable. We analyzed the two fourth-order sequences (i.e., based on the type of the current stimulus and of the preceding three; see Squires et al., 1976), which are expected to generate the lowest and the highest P300, respectively. "Continued" sequences, that is, sequences constituted by four repetitions of the same stimulus, induce the lowest P300 in response to the current stimulus, whereas "discontinued" sequences, that is, sequences where the current stimulus is preceded by three stimuli of the other type, induce the highest P300 (Jentzsch & Sommer, 2001; Squires et al., 1976). We excluded epochs that contained artifacts (ocular or other) or an interruption of the stimulus sequence, that is, a pause between blocks of trials or a recalibration of the eye tracker. The average minimum number of epochs for each participant, stimulus type, and sequence in this subset of data was 14. This number of trials was not sufficient to precisely identify peaks of microsaccade rate; consequently, we used the presence or absence of at least one microsaccade in the time WOI between 270 and 370 ms after stimulus onset as an index of microsaccadic inhibition. The mean values of the two measures for these two sequences are plotted in Figure 5. The pattern of modulation appeared to be opposite in the two measures, and the modulation was apparently stronger for the P300 measure.

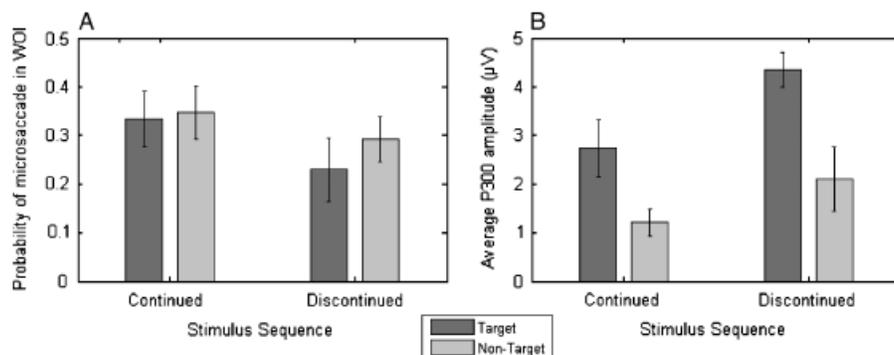
A two-way ANOVA of microsaccade rates with Stimulus Category (target vs. nontarget) and Sequence (continued vs. discontinued) as factors and the probability of occurrence of at least one microsaccade in the WOI as the dependent variable yielded a significant effect of the factor Sequence,  $F(1, 11) = 6.00$ ,  $p < .032$ , whereas the effect of the factor Stimulus Category and the interaction were not significant (both  $F_s < 1$ ). The same analysis was applied on the P300 measure and yielded a significant effect of the factor Sequence,  $F(1, 11) = 10.15$ ,  $p < .009$ , and Stimulus Category,  $F(1, 11) = 30.56$ ,  $p < .001$ , whereas their interaction was not significant ( $F < 1$ ). Once again, when the P300 was higher in amplitude for the discontinued sequences, the probability of occurrence of a microsaccade in the time WOI was lower. In addition, the P300 amplitude for target stimuli was higher as compared to nontargets, whereas no significant difference was found for the measure of microsaccadic inhibition.

The present analysis showed that sequence effects were evident in microsaccades as well as ERPs. In particular, discontinued sequences elicited a stronger microsaccadic inhibition and a larger P300 amplitude compared to continued sequences.

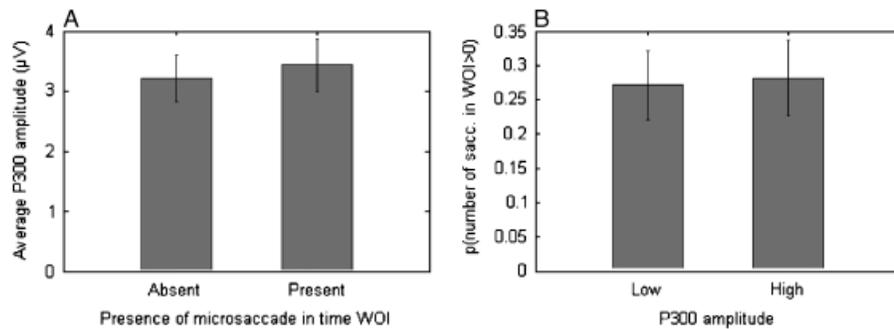
### Relation between Microsaccadic and P300 Effects

To investigate whether our measures of microsaccadic inhibition and P300 are functionally related, we addressed whether the two measures are predictive of each other at the trial level. In particular, we checked whether the observation of at least one microsaccade in the time WOI was predictive of the P300 amplitude. To this purpose, for each participant and among the subset of target trials from the 50% frequency condition, we identified the epochs in which at least one microsaccade was observed in the time WOI and epochs in which no microsaccade was observed in the same time window. This subset was chosen because we had enough trials, the amplitude of P300 to targets was large enough to allow its detection on single trials, and because all stimulus sequences occurred with the same probability. The minimum number of saccade-present epochs for each participant was 79; the minimum number of saccade-absent epochs for each participant was 12. A paired  $t$  test showed that the amplitude of P300 was not significantly higher in saccade-absent epochs than in saccade-present epochs,  $t(11) = 1.50$ ,  $p = .161$  (see Figure 6A).

We also conducted the converse analysis, that is, in the same epochs (Target and 50% Stimulus Frequency) we checked



**Figure 5.** Sequence effects on the probability of execution of a microsaccade in the time WOI (between 270 and 370 ms after stimulus onset) (A) and on the amplitude of the P300 (average ERP amplitude at P3, Pz, and P4 between 200 and 500 ms after stimulus onset) (B). The plots refer to the stimuli presented in the 50% frequency condition. "Continued" indicates a sequence of four identical stimuli and "Discontinued" indicates a sequence with three identical stimuli followed by a different one (the current stimulus). Error bars are between-participants standard errors of the mean.



**Figure 6.** A: Average P300 amplitude in 50% frequency target epochs as a function of the presence of at least one microsaccade in the time WOI between 270 and 370 ms after stimulus onset. B: Average probability of observing at least one microsaccade in the time WOI between 270 and 370 ms after stimulus onset in 50% frequency target epochs as a function of P300 amplitude (median split classification). Error bars are between-participants standard errors of the mean.

whether the measure of P300 was predictive for the execution of a microsaccade independently of stimulus frequency and stimulus category. We performed a median split of the subset of trials based on the amplitude of P300 (average voltage at Pz, P3, and P4 between 200 and 500 ms after stimulus onset). This analysis yielded a minimum number of 90 epochs per participant and P300 amplitude bin (high vs. low). A paired *t* test showed that the probability of observing at least one microsaccade in the time WOI was not significantly lower in the high-P300 epochs than in the low-P300 epochs,  $t(11) = 0.69$ ,  $p = .51$  (see Figure 6B).

## Discussion

Several studies in the last decade have shown that microsaccades can be used as a tool to investigate the state of the cognitive system (see Engbert, 2006). In particular, Valsecchi et al. (2007) showed that the rate of microsaccades presents a prolonged inhibition when a rare target is encountered in a visual oddball task.

We propose that the microsaccadic inhibition is a new tool to investigate the brain's response in the oddball task, along with the other peripheral measures that have been studied in the past decades (e.g., Bahramali et al., 1997; Friedman et al., 1973; Lyytinen et al., 1992).

An enhancement of P300 amplitude is also commonly observed in response to visual oddballs (Hermann & Knight, 2001). The amplitude of the P300 component is sensitive to the sequence of the stimuli preceding the upcoming one (Duncan-Johnson & Donchin, 1977; Jentzsch & Sommer, 2001; Squires et al., 1976), so that stimuli discontinuing the preceding sequence elicit a higher P300.

### Category/Frequency Effects on Microsaccades

The first aim of the present study was to establish whether the prolonged inhibition of microsaccades that was observed by Valsecchi et al. (2007) was due to target effects, to frequency effects, or to a combination of both. To answer this question, we conducted a visual oddball experiment varying the frequency of targets, which was 20%, 50%, and 80% in different conditions. Given the fact that the rebound in the rate of microsaccades, which normally follows the inhibition peak after the presentation of a visual stimulus (e.g., Engbert & Kliegl, 2003; Galfano et al., 2004), was clearly recognizable in the single-participant plots, we were able to individuate three measures of microsaccadic inhibition. The first measure was the rate of microsaccades in the

time window where the rebound in response to the most frequent nontargets was observed. The second measure was the latency of the rebound peak, and the third was its amplitude. The three measures were not equally sensitive to the experimental manipulations, but in general we observed a more pronounced inhibition of microsaccades in response to less frequent stimuli, and this effect was stronger for targets. Pure target effects were only observed for the latency of the rebound peak, whereas they were not significant for the other two measures.

### Category/Frequency Effects on P300

Simultaneously with microsaccades, we also recorded ERPs. We found P300 amplitude to be larger for less frequent stimuli and for targets as compared to nontargets. Moreover, the P300 amplitude modulation by stimulus frequency was stronger for targets than for nontargets, a pattern of results that has been reported previously (e.g., Duncan-Johnson & Donchin, 1977; Potts, Patel, & Azzam, 2004) and that has been interpreted as a sign of attentional effects on stimulus processing (Kok, 2001). In other words, the rare targets would capture attention more than frequent and irrelevant stimuli. In the present study, microsaccadic inhibition and P300 were modulated in a coherent way by the task relevance and by the overall frequency of the stimuli, except for the fact that the target effect was less reliable for microsaccades, being significant only when the latency of the rebound peak was taken as a measure of microsaccadic inhibition.

### Sequence Effects

We further addressed whether microsaccadic inhibition is influenced by the stimulus sequence. It has long been known that stimuli interrupting a series of identical stimuli induce a higher P300, irrespective of the a priori stimulus probability (Duncan-Johnson & Donchin, 1977; Jentzsch & Sommer, 2001; Squires et al., 1976). We replicated this observation in our P300 measure. Even when target and nontarget stimuli were equally probable overall, after three identical stimuli in a row, a stimulus change elicited a higher P300 than another repetition. This was true independently of whether the final stimulus was a target or not. Interestingly, the same pattern emerged in microsaccadic inhibition; the rate of microsaccades was more inhibited for discontinued than for continued repetition runs. As in the case of P300, the sequence effect on microsaccadic inhibition was observed both for targets and nontargets.

We can thus conclude that the overall probability and the preceding stimulus sequence determine both the amplitude of P300 and the extent of microsaccadic inhibition elicited by task-relevant stimuli. The somewhat weaker impact of task relevance on microsaccades as compared to stimulus frequency suggests that stimuli with an extremely low subjective probability could induce a microsaccadic inhibition even when task irrelevant. This, for example, could be the case of stimuli that are task irrelevant and are presented only once in the experiment and that are known to elicit the so-called novelty P300 (Courchesne, Hillyard, & Galambos, 1975; see Friedman, Cycowicz, & Gaeta, 2001). Moreover, we cannot exclude the possibility that sequence effects on microsaccadic inhibition are observed even when all stimuli are task irrelevant. However, this seems unlikely, because we have shown that, when participants passively view the stimuli, even overall rare stimuli have little effect on microsaccadic behavior (Valsecchi et al., 2007).

Notice that the ERP effects we observed are around two orders of magnitude greater than the maximum propagation of microsaccadic ocular artifacts expected at Pz. Therefore the impact of artifacts due to microsaccade-related movements of the retino-corneal dipoles on ERPs can be neglected as far as the measurement of P300 in oddball paradigms is concerned.

#### **Comparison with Previous Studies**

In the current study we confirmed that the flashing of a visual stimulus induces an inhibition of microsaccade rate with a latency between 100 and 150 ms, which is then followed by a rebound. It is interesting that the peak rate of microsaccades in response to frequent nontarget stimuli in the present study was much higher than in the study of Valsecchi et al. (2007). This might depend on the different eye-tracking systems used in the two studies. In particular, the system used in the present study would only have supported binocular recording with a lower image quality and had a lower sampling frequency, being thus more noise sensitive. Nonetheless, we basically replicated the finding that standard stimuli induce a double-phase inhibition-rebound modulation in the absolute microsaccade rate and that the inhibition phase was longer and the rebound delayed in response to oddballs. In the study by Valsecchi et al. the rebound phase was almost abolished in response to oddball stimuli, whereas in the present study it was still clearly identifiable. We suspect that this might depend on two differences between the experimental procedures. First, the frequency of rare stimuli was raised from less than 10% in the Valsecchi et al. study to 20% in the present one, thus reducing the frequency effect. Second, in the present study the stimulus series were fully randomized, whereas Valsecchi et al. used pseudorandomized series, in which at least six standard stimuli were presented between two oddballs. Hence, in the latter case the sequence effect should be much stronger than in the present case, leading to a more pronounced inhibition of microsaccades.

#### **Relationship between Microsaccadic Inhibition and P300 Enhancement**

To summarize, we showed that stimulus category, stimulus frequency and the previous stimulus sequence modulated the amplitude of P300 and that stimulus frequency and category interacted synergistically. The same effects were also observed as far as microsaccadic inhibition is concerned, with the following difference: A stimulus category effect was significant in the latency of the peak microsaccade rate, whereas the other measures

of microsaccadic inhibition only showed a reliable stimulus frequency effect. As stated above, in all of the previous studies on microsaccadic inhibition in the oddball task, it was not possible to isolate the peak microsaccade rate in response to targets. Therefore, it is still an open question whether or to what extent the different measures of microsaccadic inhibition we used in this paradigm indexed different aspects of stimulus processing in the oddball task.

A final question we addressed was whether microsaccadic inhibition and the P300 shared more than the sensitivity to the same experimental manipulations. Within a specified cell of our experimental design, the microsaccadic behavior was not significantly predictive of the P300 amplitude and vice versa. This could be due to low statistical power, given the relatively small sample size, and to the unreliability of our measures. In any case, the relationship between microsaccadic inhibition and P300 enhancement deserves further study, which could either reveal a relation that went undetected in our experiment or prove the independence of these two measures of the brain's response to rare targets in a visual oddball task.

The debate over the functional meaning of P300 has continued for four decades since this component was first reported (Sutton, Braren, Zubin, & John, 1965). In general, the most accepted view is the one that P300 is an index of context updating (Donchin, 1981; Donchin & Coles, 1988). This theoretical perspective considers P300 as a sign of the attentive restructuring of the stimulus representation in working memory when a new stimulus is encountered. Over the years, extensive evidence regarding the neural processes that could support the attention and memory operations related to P300 generation has been collected. Overall, the data seem compatible with the hypothesis that P300 reflects the neural inhibition that is functional to the focusing of activity on the processing of target stimuli (Polich, 2007). The inhibition model proposed by Polich is more specific in that it posits that the inhibition is revealed by a P3a when focal attention is summoned by rare distractors and revealed by a P3b when targets are evaluated in working memory. As far as the current evidence is concerned, we can propose that a similar mechanism also subtends the inhibition of microsaccades elicited by oddball stimuli; it is in fact clear that the inhibition of microsaccades is observed when a stimulus that requires a deeper restructuring of the task-related representation is encountered. However, in the present experiment we did not present rare distractors together with rare targets and frequent standards (three-stimulus oddball paradigm), which is the experimental paradigm where P3a and P3b are more clearly distinguishable. Therefore, we cannot specify whether microsaccadic inhibition is more related to one of the two components.

A deeper knowledge of the neural system involved in the generation of microsaccades and in their inhibition could also probably be helpful in disentangling the differences between this oculomotor effect and the enhancement of P300 observed in response to infrequent targets in the oddball task. The neural generators of P300 have been studied using intracranial recordings (Halgren et al., 1995a, 1995b, Halgren, Marinkovic, & Chauvel, 1998; Roman, Brázdil, Jurák, Rektor, & Kukleta, 2005). A widespread network of cortical areas in the parietal, frontal, and temporal lobes and subcortical areas such as the hippocampus and the amygdala were identified as generators of P3-related ERPs. These findings have been confirmed by fMRI studies (Ardekani et al., 2002; Bledowski, Prvulovic, Hoehstetter, et al., 2004; Bledowski, Prvulovic, Goebel, Zanella, & Linden, 2004;

Clark, Fannon, Lai, Benson, & Bauer, 2000; Stevens, Skudlarski, Gatenby, & Gore, 2000). As far as microsaccades are concerned, there is indirect evidence that they are triggered by fixational activity within the superior colliculus, mainly derived from the observation that saccades, which are known to be elicited by stimulation of the superior colliculus (Robinson, 1972), feature a kinematic profile similar to the one of microsaccades (Zuber et al., 1965). Furthermore, it has been demonstrated that the inhibition of microsaccades in response to visual stimuli can be mediated by a cortical visual pathway sending afferences to the superior colliculus (Valsecchi & Turatto, 2007). Therefore we cannot exclude that some of the cortical generators of P300

could also be responsible for the prolonged inhibition of microsaccades. Overall, the current neurophysiological evidence does not indicate that the structures generating microsaccades and the P300 are anatomically segregated. It is well possible that the prolonged microsaccadic inhibition reflects inhibitory processes within cortical oculomotor areas, a mechanism similar to the one that has been proposed for P300 (Polich, 2007).

To conclude, we propose that P300 enhancement and prolonged microsaccadic inhibition are both indices of the brain's processing of subjectively rare relevant stimuli. Further research is needed to clarify the extent to which these two measures are functionally related.

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