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**Analysis of microsaccades during extended practice of a visual discrimination
task in the macaque monkey**

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1 **Abstract**

2 The spatial location indicated by a visual cue can bias microsaccades directions towards or
3 away from the cue. Aim of this work was to evaluate the microsaccades characteristics during the
4 monkey's training, investigating the relationship between a shift of attention and practice. The
5 monkey was trained to press a lever at a target onset, then an expanding optic flow stimulus
6 appeared to the right of the target. After a variable time delay, a visual cue appeared within the
7 optic flow stimulus and the monkey had to release the lever in a maximum reaction time (RT) of
8 700 ms. In the control task no visual cue appeared and the monkey had to attend a change in the
9 target color. Data were recorded in 9 months. Results revealed that the RTs at the control task
10 changed significantly across time. The microsaccades directions were significantly clustered
11 toward the visual cue, suggesting that the animal developed an attentional bias toward the visual
12 space where the cue appeared. The microsaccades amplitude differed significantly across time. The
13 microsaccades peak velocity differed significantly both across time and within the time delays,
14 indicating that the monkey made faster microsaccades when it expected the cue to appear. The
15 microsaccades number was significantly higher in the control task with respect to discrimination.
16 The lack of change in microsaccades rate, duration, number and direction across time indicates that
17 the experience acquired during practicing the task did not influence microsaccades generation.

18 **Introduction**

19 The sensory systems continuously receive signals, which cannot be simultaneously
20 analyzed at the conscious level. Raja Parasuraman [18] identified three different components of
21 attention: 1) selective attention (or selection), which determines the processing of peculiar
22 information; 2) vigilance, the ability to maintain attention over time; 3) control, the ability to plan
23 and coordinate different activities. Attentional processes vary in relation to the object of attention,
24 the way in which attention is expressed and the time periods in which these mechanisms occur.
25 Spatial attention represents a submodality of attention, which participates in all attentional
26 processes. Spatial attention allows the observer to selectively analyze visual information,
27 prioritizing a specific region of the visual field; information coming from the specific region then
28 receives further processing.

29 Our eyes are never still. Small eye movements, called, microsaccades, change the position
30 of the eyes [15]. Previous studies linked microsaccades to perception [14, 16] and determined the
31 key interactions between the dynamics of microsaccades and cognitive processes, especially
32 regarding the location of attention [5, 8, 20-23]. The spatial position indicated by an attentional
33 signal can influence the direction of the microsaccades towards or away from that signal:
34 endogenous stimuli tend to shift attention towards the signal, while exogenous stimuli tend to
35 remove it [7].

36 The relationship between attention and microsaccades has been extensively studied both in
37 human subjects and in non-human primates using attentional, cued or discrimination tasks.
38 However, little is known about the time course of the microsaccades during the entire process of
39 learning and practicing a visual task that requires the orientation of attention. In the last decade, a
40 number of studies showed that specific conditions modulate microsaccades, like exogenous

41 emotional stimuli [11], multisensory integration [33] and cognitive processing load [4, 6]. These
42 studies encouraged us to evaluate if the consolidation of a learning process, which requires different
43 cognitive processing loads across time, influences the microsaccades generation.

44 **Materials & Methods**

45 The experiments were performed on one male macaque monkey (*M. fascicularis*). All
46 experimental procedures received the approval of the Committee on Ethics in Animal
47 Experimentation of the University of Bologna and of the Italian Ministry (approval n. 258/2015-
48 PR).

49 Surgical procedures

50 Before the beginning of the training, three metal skull pins for restraining the head were
51 implanted by using titanium screws. Surgical procedures were performed under general anesthesia
52 (Thiopentotal Sodium 15 mg/Kg i.v.). Analgesic and antibiotics were given for several days after
53 surgery (Ketorolac tromethamine, 30 mg/die i.m.; Benzathine Benzylpenicillin 0.1 ml/Kg i.m.)
54 following the same protocol used in previous studies [25-30]. During the experiments, the head
55 was restrained using a halo head fixation system similar to that describer by Azimi et al. [1]. After
56 recovering from surgery, the monkey started the training procedure.

57 Experimental paradigm

58 The monkey sat in a primate chair in front of a TV monitor covering 62 x 51° of visual
59 field. The screen was placed 57 cm from the monkey's eyes. The monkey was trained in looking
60 at a fixation point (FP) formed by a red square (0.5° side), in a visual discrimination task. At the
61 FP onset, the monkey pressed a lever and an expanding optic flow stimulus appeared to the right
62 of the fixation point. After a variable delay of 750, 1000, 1250 or 1500 ms, a visual cue appeared
63 within the optic flow stimulus and the monkey was trained to release the lever in a maximum RT

64 of 700 ms. The optic flow stimulus was 8° in radius and the focus of expansion was positioned 15°
65 of eccentricity from the FP. The visual cue was a small sphere composed of dots, which moved
66 randomly within the sphere (Figure 1A). The sphere used as visual cue had a radius of 2° . The
67 random movement of the dots made the sphere very visible because the dots of the optic flow
68 stimulus moved in radial expansion. We used a control task in which no visual cue appeared within
69 the optic flow stimulus and the monkey was trained to attend a change in the target color (Figure
70 1B). To avoid the effect of timing, the recordings were performed in random order. Visual stimuli
71 were generated using the Matlab Psychophysical Toolbox. A drop of water was given as a reward
72 for each correct trial. Eye movements were recorded binocularly by the EyeLink II (SR Research
73 Ltd, Mississauga, Canada). Pupil tracking was performed at 500 samples/s, with a gaze resolution
74 $<0,005^\circ$ and noise limited to $<0.01^\circ$. Trials with eye positions outside a 1° window around FP were
75 discarded.

76 Data analysis

77 The RTs related to the control and discrimination task were analyzed for each data set. To
78 analyze the potential RT changes through the entire training period of nine months, we divided the
79 RT values into six epochs. Each epoch contained the same number of data (i.e. training days)
80 corresponding to about 5-6 weeks of training. The monkey's RTs have been analyzed using a
81 repeated measure analysis of variance (ANOVA) having the RTs of time delay (750, 1000, 1250,
82 1500 ms), task (discrimination, control) and epochs (month 1-9) as within subject factors. The data
83 were analyzed with the SPSS v22.0 statistical software (SPSS, Chicago, IL, USA).

84 Microsaccades are defined as eye movements with an amplitude less than 1° and with a peak
85 velocity up to $100^\circ/s$ [34]. For the identification of microsaccades we used the algorithm of Engbert
86 and Kliegl [5]. Partial and complete pupil occlusions, including the previous and subsequent 200

87 ms from the occlusion, were discarded. Microsaccades amplitude, peak velocity, number, rate,
88 duration and direction were calculated and averaged in each task and in each time delay of each
89 experiment. The frequency of the microsaccades was calculated considering only the time spent
90 during the fixation period, dividing the number of microsaccades by the duration of the recording
91 in seconds [17]. The potential variations of microsaccades across the entire period of training were
92 assessed on each parameter (amplitude, peak velocity, number, rate and duration) using a repeated
93 measure ANOVA having the time delay, task and epochs as within-subject factors.

94 The relationship between microsaccades amplitude and peak velocity (the main sequence),
95 and the correlation between RT changes and variations of microsaccades across the six epochs
96 were assessed using the Pearson correlation coefficient.

97 Microsaccades directions were analyzed in each epoch using circular statistics (Oriana®
98 4.0 for Windows, Kovach Computing Services, Anglesey, Wales). The uniformity of the mean
99 vectors distribution was assessed with the Rayleigh test.

100 **Results**

101 Reaction times

102 Results of the repeated measure ANOVA on the RTs showed a significant main effect for
103 task ($F_{1,1} = 777.66$; $p < 0.001$; $\eta_p^2 = 0.985$), time delay ($F_{1,3} = 26.63$; $p < 0.001$; $\eta_p^2 = 0.685$) and
104 epochs ($F_{1,5} = 6.002$; $p < 0.001$; $\eta_p^2 = 0.333$). Moreover, significant interaction effects were
105 observed for task x time delay ($F_{1,3} = 16.77$; $p < 0.001$; $\eta_p^2 = 0.583$), task x epochs ($F_{1,5} = 10.812$;
106 $p < 0.001$; $\eta_p^2 = 0.474$), time delay x epochs ($F_{1,15} = 4.353$ $p < 0.001$; $\eta_p^2 = 0.266$) and for all factors:
107 task x time delay x epochs ($F_{1,15} = 3.582$ $p < 0.001$; $\eta_p^2 = 0.23$) (Figure 2). The Bonferroni pairwise
108 comparison showed that the RTs in the visual discrimination task were significantly longer than
109 those of the control task ($p < 0.001$). Regarding the time delay, the Bonferroni pairwise comparison

110 showed that the RTs of the 750 ms (the shorter fixation period) were significantly different than
111 those of the other fixation times (750 vs 1000 $p = 0.003$; 750 vs 1250 $p < 0.001$; 750 vs 1500 $p <$
112 0.001). Regarding the epochs, significant differences were found between epochs 1 and 3 ($p =$
113 0.003), epochs 2 and 3 ($p = 0.001$) and epochs 3 and 6 ($p = 0.033$).

114 During the training, the monkey made very few errors. In the 750 ms delay, the correct
115 trials were 6901 (92.5%), while the early releases of the key were 147 (2%) and the late releases
116 405 (5.5%). In the 1000 ms delay, the correct trials were 5992 (93%), the early releases 286 (4%)
117 and the late releases 172 (3%). In the 1250 ms delay, the correct trials were 4956 (88%), the early
118 releases 581 (10%) and the late releases 118 (2%). In the 1500 ms delay, the correct trials were
119 4476 (77.5%), the early releases 1185 (20.5%) and the late releases 103 (2%). The only notable
120 result is in the increase in the early release of the key that increases with the increment of the time
121 delay.

122 Microsaccades

123 The microsaccades main sequence, i.e. the relationship between amplitude and peak
124 velocity, showed that the monkey made very fast microsaccades with large amplitude
125 corresponding to the stimulus eccentricity (Figure 3). Analysis showed positive correlation
126 coefficients between amplitude and peak velocity for both discrimination ($r = 0.53$, $p < 0,001$) and
127 control task ($r = 0.52$, $p < 0,001$).

128 Results of the repeated measure ANOVA on the microsaccades peak velocity (Table 1)
129 showed a significant main effect for epochs ($F_{1,5} = 9.43$; $p < 0.001$; $\eta_p^2 = 0.35$) and time delay ($F_{1,3}$
130 $= 4.94$; $p = 0.004$; $\eta_p^2 = 0.22$) and a significant interaction effect of epochs x time delay ($F_{1,15} =$
131 2.19 ; $p = 0.007$; $\eta_p^2 = 0.11$). The Bonferroni pairwise comparison on the time delays showed few
132 differences in the microsaccades peak velocity (1000 vs 1500 $p = 0.035$; 1250 vs 1500 $p = 0.049$).
133 Regarding the epochs, significant differences were found between epochs 1 and 2 ($p = 0.048$),

134 epochs 2 and 4 ($p = 0.009$), epochs 2 and 5 ($p = 0.037$), epochs 4 and 6 ($p = 0.005$) and epochs 5
135 and 6 ($p = 0.001$).

136 The analysis of the microsaccades amplitude (Table 1) showed a significant main effect for
137 epochs ($F_{1,5} = 6.97$; $p < 0.001$; $\eta_p^2 = 0.29$). The Bonferroni pairwise comparison showed that all
138 epochs differed significantly from each other (all significant values were smaller than 0.007).

139 The analysis of the microsaccades number (Table 1) showed a significant main effect for
140 condition ($F_{1,1} = 50.16$; $p < 0.001$; $\eta_p^2 = 0.74$).

141 No significant differences were found for microsaccades rate and duration.

142 Results of the circular statistical analysis showed that microsaccades directions were
143 significantly clustered toward the optic flow stimulus in all 6 epochs of the training period (Figure
144 4). The Rayleigh test of uniformity showed non-uniform significant distribution for both
145 discrimination and control task ($p < 0.001$).

146 Correlation between microsaccades and RTs

147 We correlated the microsaccades values with the RTs at the discrimination task in the 6
148 epochs. In the 750 ms delay, in epoch 1 RTs were correlated with microsaccades duration ($r = 0.56$,
149 $p = 0.049$), while in epoch 6 RTs were correlated with both amplitude ($r = 0.69$, $p = 0.009$) and
150 duration ($r = 0.75$, $p = 0.003$). In the 1000 ms delay, in epoch 1 RTs were correlated with
151 microsaccades number ($r = 0.62$, $p = 0.022$). In the 1250 ms delay, in epoch 3 RTs were correlated
152 with microsaccades amplitude ($r = 0.68$, $p = 0.01$). In the 1500 ms delay, in epoch 1 RTs were
153 negatively correlated with microsaccades amplitude ($R = -0.62$, $p = 0.022$) and rate ($r = -0.63$, $p =$
154 0.018), while in epoch 6 RTs were negatively correlated with microsaccades rate ($r = -0.71$, $p =$
155 0.006).

156 **Discussion**

157 Behavioral reaction times

158 The RTs in the control task were constantly shorter than those of the discrimination task,
159 indicating that the perceptual processing of shifting attention toward the visual cue lasts about 150-
160 200 ms. Results show that the monkey's strategy was very specific: we observed the longest RTs
161 in the discrimination task during the shorter time delay (750 ms). RTs then reduced in all other
162 conditions with longer cue presentation time suggesting that the monkey did not pay attention to
163 the optic flow stimulus from the beginning of the task, but oriented its attention later in time.

164 The sudden onset of a visual stimulus draws attention toward the stimulus location
165 enhancing perceptual sensitivity [19]. Krishna et al. found that manual reaction times on a detection
166 task were slower when the attentional cue appears at a saccade goal [13]. In our study, eye
167 movements outside the tolerance window of 1° caused the end of the trial, so the monkey was
168 prevented to make a saccade toward the optic flow stimulus. However, the monkey's attentional
169 requirements could have inhibited the eye-movement generation causing an increase in the
170 attentional processing, and thus an increase in reaction times. This potential inhibitory process
171 might be linked with the inhibition of return, where manual reaction times are slower in response
172 to stimuli presented at previously cued locations [7, 12, 24]. We point out that the monkey
173 continuously shifted its attention to the previous cued location, so that the slowdown of RTs in the
174 shorter conditions can be explained by an inhibitory effect resulting from the necessity of
175 suppressing the natural tendency to make a saccade toward a cue [2, 3, 12].

176 The concept of attention is very complex as it involves many multi-channel sensorimotor
177 systems [10]. The attentional strategy used by our monkey represents a good example of different
178 sensory-motor processing, as it was slower with shorter presentation times and faster with longer
179 presentation times. In our protocol, the monkey did not know if the trial would be discrimination
180 or control. We see that the monkey always made microsaccades toward the optic flow stimulus but

181 the RTs analysis seems to indicate that the change in color of the fixation point (from red to green)
182 was a much stronger stimulus for lever release than the visual cue. Presumably, the monkey's
183 attention was focused on the fixation point and the monkey continuously changed its strategy
184 shifting attention to the optic flow stimulus to be able to perform the discrimination task. As already
185 suggested by Krishna et al. [13], this strategy change could involve a greater engagement of
186 endogenous attention.

187 Microsaccades characteristics

188 Microsaccades rate, duration, direction and number did not vary across the nine months of
189 recordings, likely indicating that the monkey, to discriminate the stimulus, established a strategy
190 at the beginning of the training, keeping it for the whole practice process. The lack of change in
191 microsaccades production could be explained by the microsaccadic inhibition. This phenomenon
192 was initially observed after the presentation of attentional cues, but it is now well known that
193 microsaccades generation is affected by any transient stimulus, like the sudden appearance of an
194 object or the change in contrast [5, 8, 31, 32]. Further, visual stimulus features can modulate the
195 duration of the inhibition and/or the strength and latency of the subsequent rebound [9].

196 The time since the last microsaccade is indicative of the likelihood of an upcoming
197 movement [9]. Using a neural network model, Hafed and Ignashchenkova explained that
198 microsaccades can occur at specific phases of ongoing physiological α rhythms and these
199 movements also exhibit signatures of oscillatory behavior. When such a rhythm is phase reset by
200 stimulus onsets then the classic microsaccadic rate signature would be expected [9]. The stimuli
201 used in the present study were presented to the monkey for nine months. It is possible to
202 hypothesize that the microsaccades production did not change across time due to the identical
203 stimulus features, which could have made such oscillatory behavior very constant. Future study

204 could be designed to confirm this hypothesis changing the stimuli features, like contrast, spatial
205 position (i.e. left-right or up-down) and optic flow direction. In this study protocol, we only used
206 one optic flow stimulus without varying the focus of expansion eccentricity, the spatial position
207 and the dot speed because changing such features would have complicated the monkey's training
208 resulting in a misleading interpretation of the results.

209 The analysis of the main sequence showed that the monkey made very fast microsaccades
210 with large amplitude corresponding to the stimulus eccentricity. Amplitude and peak velocity
211 showed significant differences across the entire period of recordings, however, the microsaccades
212 directions were clustered where the visual cue was expected to appear. This is in line with the
213 strategy to make faster microsaccades when the monkey expected the cue to appear.

214 The microsaccades number was significantly higher in the control task in all conditions.
215 This is interesting, as the monkey did not know if the trial would be discrimination or control until
216 the cue onset or the change in target color. It is possible to hypothesize that the monkey focused
217 more on the fixation point making more microsaccades toward the optic flow stimulus.

218 Lastly, the circular analysis showed that microsaccades directions have been clustered
219 toward the optic flow stimulus for the entire training process. Such spatial asymmetry of the
220 microsaccades suggests that the animal developed an attentional bias towards the place where the
221 cue appeared in some trials. This result confirms that microsaccades directions could indicate the
222 position of the attentional focus without significant variations during the training process. Note that
223 the monkey was trained to press/release the lever with both hands, so to avoid any potential shift
224 of attention due to the arm movement.

225 **Conclusions**

226 This study aimed at uncovering the relationship between microsaccades generation,
227 attention and training. Results showed that the monkey was more focused on the fixation point,
228 producing more microsaccades in the control task. The experience acquired during training did not
229 change the microsaccades production, indicating that practicing a visual discrimination task does
230 not affect microsaccadic circuitry. The correlation analysis showed very few significant
231 relationships between RTs and microsaccades, confirming that the developed behavioral strategy
232 and microsaccades generation seem to be independent processes.

233 **Acknowledgements**

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235 the head holder system. This work was supported by University of Bologna.

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310

311

312 **Figure legends**

313

314 **Figure 1.** Task. **A.** Visual discrimination task. **B.** Control task. Note that the figure is not to scale,
315 see Methods for stimuli and fixation point dimensions.

316

317 **Figure 2.** Frequency histograms of reaction times for both visual discrimination (grey) and control
318 task (black) in each time delay in all epochs. Data are reported as mean values \pm SE. **A.** Cue
319 appeared after 750 ms from the target onset. **B.** Cue appeared after 1000 ms. **C.** Cue appeared after
320 1250 ms. **D.** Cue appeared after 1500 ms. Asterisks indicate significant effects (see Results for
321 descriptions).

322
323 **Figure 3.** Main sequence of the microsaccades. **A.** Visual discrimination task. **B.** Control task.

324
325 **Figure 4.** Distributions of microsaccades directions in all epochs during the training period. **A.**
326 Visual discrimination task. **B.** Control task. Mean vectors are computed for each trial. Solid line
327 crossing each diagram indicates the significant mean vectors, curved line outside the circle indicate
328 the circular SD. For each epoch $n = 68$. Bars are 20° width.

329
330 **Table 1.** Mean values of microsaccades parameter that showed significant differences across time
331 or epochs. Data are shown as mean value \pm SE (see Results for description of significant values).