The Spatiotemporal Characteristics of Microsaccade Reflect the Interaction between Saccadic Choice and Intrinsic Directional Bias

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Abstract

Microsaccades have long been reported to associate with visual perception and other cognitive functions such as eliminating visual fading, supporting detailed visual sampling and indicating the direction of covert attention. However, despite the knowledge that the direction of microsaccades might be modulated by multiple factors, e.g., the loci of covert attention and saccadic target, the detailed modulations remain unclear due to the fact that the spatiotemporal dynamics of microsaccades direction has not been systematically studied. The specific questions include: 1) Is the change of microsaccades direction in a saltatory or continuous manner? 2) Does the change of microsaccades direction reflect the real-time information processing in brain? To address such questions, it is significantly important to elaborately study the directional characteristics of microsaccades along time in behavioral tasks, which will enable us to unveil the task relevance of microsaccades direction. Here we report that, in a spatial cue instructed saccade task where there were two competing saccadic choices separated in space, the direction of microsaccades of two monkeys started to rotate shortly after the onset of the spatial cue until the onset of the go-signal for a responsive saccade. Moreover, the speed of the directional rotation varied between two different choices, which was attributed to monkeys’ internal bias. However, the rotation of microsaccades direction was absent in a classical memory-guided saccade task. These results indicate that, in choice task condition, the direction of microsaccades changed continuously that reflects the real-time interaction between saccadic choice and internal directional bias.

Keyword: rotation; microsaccades; spatial choice; inner bias

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Introduction

Microsaccades are small fixational eye movements, which was first empirically identified by Robert Waring Darwin (the father of Charles Darwin) in 1786 [1, 2]. From the early 1950s to the early 1980s, there was a debate on whether microsaccades play a functional role in counteracting visual fading [3-5] or serve no significant functions [6-14]. Until year 2006, Martinez-Conde et al. reported that microsaccades played a causal role in counteracting peripheral visual fading (Troxler’s effect) [15], which was confirmed by later studies [16]. Farther more, microsaccades were also found to associate with other brain functions such as supporting visual information sampling near the fovea [17-22] and indicating the loci of covert attention [23-29].

As one of the most important metrics of microsaccades, the direction of microsaccades were reported to be modulated by various activities, such as perceiving visual details near the fovea [18, 20], orienting covert attention [23-29], choosing saccadic target [30] and expecting reward size [30]. However, these studies either only focused on study of microsaccades direction in discrete time points or on analysis of microsaccades direction in very low spatial resolutions, and yet missed the detailed dynamics of microsaccades direction in both spatial and temporal dimensions. The specific questions include: 1) Is the change of microsaccades direction in a saltatory or continuous manner? 2) Does this change of microsaccades direction reflect the real-time information processing in brain?

To investigate these questions, we analyzed the direction of microsaccades of two macaques while they were performing two oculomotor tasks. In a spatial cue instructed saccade task (SCIS task), monkeys needed to choose one from two saccadic targets as the saccadic goal based on the spatial location of a visual cue (left visual field versus right visual field). Intriguingly, the direction of microsaccades of these two monkeys started to rotate soon after the spatial cue onset and continued until a responsive saccade was allowed. The rotation speed of microsaccades direction differed between two different saccadic choices, and it was significantly larger when the direction of saccadic choice was opposite to monkey’s intrinsically preferred direction. In contrast, no rotation of microsaccades direction was observed in a standard memory guided saccade task (MGS), in which no competing choices were presented. Thus the rotation of microsaccades direction was crucially associated with the process of saccadic choice. We propose a conceptual model to explain the involvement of the interaction between saccadic choice and intrinsic directional bias in modulation of microsaccades direction.

However, the rotated direction of microsaccades in the SCIS task did not correlated with the location of exogenous visual stimuli or the direction of endogenous saccadic choice. Such results are inconsistent with previous findings, e.g., the direction of microsaccades was correlated with covert attention [23-29] and saccadic choice [30], thus indicate that the modulation of microsaccade direction is underlying more sophisticated mechanisms.
Materials and Methods

Experimental Setup

All experiments were carried out in a dark room, and all visual stimuli were back-projected (View Sonic, PJ7383) on a large screen which covered a visual angle of 82° and was 80 cm away from monkeys’ eyes. Experimental processes were controlled using a REX system (NIH, Bethesda, MD) running on a QNX operating system. A scleral search coil system (Sclera Track 4000, Cris Instrument Co., Inc.) was used to monitor horizontal and vertical eye position at a sampling rate of 1 kHz.

Animal Preparation

The experiments were conducted on two (Abao and Datou) adult male rhesus macaques that weighted 6-8 kg and aged 6-7 years. For each monkey, a head post was surgically implanted for head fixation and a scleral search coil for eye position monitoring [31]. All experimental and surgical procedures were standard and approved by the Animal Care Committee of Shanghai Institute of Biological Sciences, Chinese Academy of Sciences and the Animal Care Committee of Beijing Normal University.

Spatial Cue Instructed Saccade Task

In the spatial-cue instructed saccade task (SCIS task, Fig. 1A), the monkeys needed to choose one out of two candidate goals as the saccadic target according to the location of a spatial cue. Each trial started with the onset of a central fixation dot on which the monkeys had to fixate until its disappearance. 500ms after attainment of fixation, two candidate saccadic targets appeared at opposite locations which were 10° to the left and right of fixation. After 400ms, a spatial cue was turned on for 200ms at a location randomly selected from a 4 × 6 matrix of positions (horizontal: ±4°, ±8°, ±12°; vertical: ±5°, ±10°) centered at the fixation dot. Then as the fixation dot disappeared after a 400ms delay period, the monkeys needed to make a saccade towards the target which was on the same side of the screen as the spatial cue.

Memory Guided Saccade Task

In the memory guided saccade task (MGS task, Fig. 1B), the monkeys needed to
make a saccade to a memorized location. Each trial began with the appearance of a central fixation dot, followed by a 500ms fixation period. Then the target was shown for 600ms, after which there was a delay period of 1000ms. At the end of the delay period, the fixation dot was removed and the monkeys had to make a saccade to the location where the target was previously displayed. There were 8 possible target locations which were evenly distributed around the fixation dot with the same eccentricity of 10°, and each trial selected one location under a uniform distribution.

**Saccade Detection and Classification**

Saccades were detected mainly through four steps. Firstly, horizontal and vertical eye positions were smoothed along time with a Gaussian convolution \((radius = 500, \sigma = 31.64)\), which removed all high frequency noises with small amplitude. Secondly, the smoothed eye trace was differentiated to obtain horizontal and vertical velocities which were then combined to calculate the overall speed, and each bump on the speed curve above the threshold of \(2.1°/s\) was regarded as a saccade candidate. Thirdly, for each candidate, two time points with \(1/3\) of the peak speed were identified on two sides of the peak respectively, and the ratio between the time intervals from these two points to the peak was computed and all candidates with this ratio less than 0.75 or larger than 1.25 were eliminated. This operation was used to clear some low-frequency asymmetric noises. Finally, the briefly smoothed eye trace (horizontal and vertical eye positions were averaged with a 11-ms sliding window for 5 times) was used to refine the initial and off time of each saccade based on a velocity threshold of the larger one between 5% of the peak velocity and \(3°/s\). Fig. 2 shows the density distribution of the main sequence [32] for two monkeys in two tasks.

Since there is a strong inhibition of the generation of microsaccades about 100ms after cue onset (Fig. 3), the time period used for rotation analysis (testing period) was from 30 ms after the time of the lowest microsaccades rate following cue onset (or target onset for the MGS task) to 600 ms after cue onset (or 1200ms after target onset for the MGS task). To be specific, this period was from 136 ms after cue onset and 152 ms after target onset for monkey Datou in the SCIS task and the MGS task respectively, while 115 ms and 129 ms for monkey Abao.

We then compared the directions of two consecutive fixational saccades during the testing period, and it was found that a large portion of fixational saccades were directed opposite to their preceding ones (see in Fig. 4 those points falling around dashed lines), might form square-wave-jerks [33, 34]. Thus, in order to remove the effect of this opposite style on the dynamics of microsaccades direction, only first fixational saccades during the testing period were considered as microsaccade candidates for the analysis of the dynamics of microsaccades direction, as what previous study did [27].

Since the size of microsaccades is more or less artificially defined in the existing literature on microsaccades [2, 35], we used a typical threshold of \(1°\) [23, 27, 28, 36-]
38], namely, only fixational saccades within 1° were classified as microsaccades.

**Rotation Measurement of Microsaccades Direction**

For analysis of rotation of microsaccades direction in the SCIS task, only upward (0°~180°) microsaccades during the testing period were used for monkey Datou, while only downward (180°~360°) ones were used for monkey Abao. The reason is that a great majority of microsaccades were upward during the testing period for monkey Datou (95.65% when chose left and 89.88% when chose right) while downward for monkey Abao (83.40% when chose left and 77.22% when chose right) (see Fig. 5).

The average dynamics of microsaccades direction were computed using a sliding time window of 50 ms and the mean direction with SEM of microsaccades within this window were calculated as the average microsaccades direction of the time point at the window center. To be noticed, the last 100 ms of the testing period were excluded from the analysis of average microsaccades direction, otherwise the microsaccade rate was too low during this late delay interval to reliably compute the average microsaccades direction.

The rotation speed of microsaccades direction was defined as the slope obtained by linear regression on data points representing microsaccades direction and occurring time. To compare speeds of microsaccades direction rotation under two different conditions, data under each condition was first arranged following data collection time and then separated into eight subgroups balanced in number of microsaccades (or seven subgroups for error trials from monkey Datou in order to obtain enough data for each subgroup). After that, the speed of each subgroup was measured using linear regression, and finally, a two-tailed permutation test was applied to test the difference between two sets of rotation speeds from these two different conditions.

**Measurement of Inner Bias**

We determine monkeys’ inner bias in the SCIS task by analyzing direction bias of break saccades (saccades go out of a checking window when keeping fixation required) made by monkeys during periods from fixation on to candidate goals on and from candidate goals on to cue on. A leftward (or rightward) break saccade is identified as going at a direction within a ±20° window centered at leftward (or rightward). For both monkeys, break saccades during those two periods were collected separately for each month, and then proportions towards left and right were computed respectively and compared by a two-tailed permutation test.
Results

Rotation of Microsaccades Direction during Delay Period Observed in the SCIS Task but Not in the MGS Task

To explore the spatiotemporal characteristics of microsaccades, a heat map was used to plot the distribution of microsaccades in which the 2D space was composed of direction and time of microsaccades (SCIS task, Fig. 6A, 6C; MGS task, Fig. 6B, 6D). To be noticed, during the testing period (from 30 ms after the cue induced lowest microsaccades rate to fixation offset, see Materials and Methods in details), only the first microsaccade of each trial was included in this analysis. About 200 ms before cue (SCIS, Fig. 6A, 6C)/target (MGS, Fig. 6B, 6D) onset, the direction of most microsaccades was upward (around $90^\circ$) for both monkeys. However, shortly after the cue/target onset, the spatiotemporal distribution pattern of microsaccades was dramatically different between SCIS and MGS tasks. In the SCIS task, the directions of microsaccades were distributed coherently within any given time window for both monkeys. Impressively, the overall direction of microsaccades for both monkeys started to gradually change shortly after the spatial cue onset until fixation offset, and the gradual change of microsaccades direction exhibited as a direction rotation without large fluctuations. Then we performed a linear regression on microsaccades during the testing period for both monkeys in the SCIS task, and used the slope of the linear regression as a measurement of the rotation speed of microsaccades direction. According to the results, for monkey Datou, when he chose the target on the right side, the direction of the microsaccades rotated clockwise from upward (about $90^\circ$) towards rightward ($0^\circ$) ($speed = -77.5^\circ/s$, $r = -0.285$), while the direction rotated clockwise very little when it chose the target on the left side ($speed = -16.0^\circ/s$, $r = 0.070$); when monkey Abao chose the target on the left side, the direction of microsaccades rotated anticlockwise from the left lower quadrant towards the right lower quadrant ($speed = 133.1^\circ/s$, $r = 0.402$), while the direction only rotated a little bit anticlockwise when it chose the target on the right side ($speed = 38.2^\circ/s$, $r = 0.121$).

These results show that microsaccades direction does change during the delay period when monkeys perform the SCIS task and this direction change is not saltatory but continuous. However, is this direction rotation of microsaccades associated with factors specific to the SCIS task or with general factors across different behavioral tasks such as loci of attention, memory maintaining and motor planning? To answer this question, we applied the same methodology to analyze microsaccades data from both monkeys in the MGS task (Fig. 1B). In contrast, data from the MGS task show very different spatiotemporal distribution patterns of microsaccades, which shows no sign of direction rotation (Fig. 6B, 6D). In detail, instead of coherent distribution of microsaccades direction in the SCIS task, the directions of microsaccades in the MGS task were diffused.
in more than half of the whole visual field, therefore, it is not viable to find a rotation pattern. In conclusion, common factors involved in both the SCIS task and the MGS task, such as the loci of attention, memory maintaining and motor planning, cannot explain the direction rotation of microsaccades; instead, the direction rotation of microsaccades should be specifically associated with factors involved in the SCIS task, e.g., alternative saccadic choices.

**Rotation Speed of Microsaccades Direction Reflected the Interaction between Saccadic Choice and Intrinsic Directional Bias of Monkeys**

An unexpected and significant observation is that the degree of directional rotation of microsaccades is very different between conditions of two alternative choices for both monkeys. Such variation cannot be explained by the effect of exogenous factors because the sensory stimulations in the SCIS task are symmetric between two choices (left versus right). Thus, the possible reason that causes the rotation difference might be the intrinsic directional bias of monkeys.

Here, we employed the saccadic direction of breaking fixation trials as a behavioral probe to assess the intrinsic directional bias of each monkey. Specifically, we analyzed the direction of breaking fixation saccades during two periods prior to the onset of cue: from fixation onset to saccadic targets onset (initial fixation period); and from saccadic target onset to cue onset (after saccadic target period). For each monkey, we first compared the proportion of breaking fixation saccades (data were grouped monthly) toward left versus toward right, and results are shown as scatter plots (left panels of Fig. 7A, 7B, 7C, 7D). Then, a single-tailed paired Wilcoxon signed rank test was performed between proportions of break saccades towards left and right. It was found that break saccades during both periods had significant higher proportions of going left than going right for monkey Datou ($p = 0.016$, $p = 0.012$), while the proportions were in an opposite way for monkey Abao ($p = 0.002$, $p < 0.001$) (right panels of Fig. 7A, 7B, 7C, 7D). Therefore, it is reasonable to infer that monkey Datou was intrinsically biased towards left while monkey Abao was intrinsically biased towards right.

Did monkey’s intrinsic directional bias affect the rotation of microsaccades? To answer this question, we divided all correct trials into two groups according to the congruency between monkey’s saccadic choice and intrinsic directional bias, that is, in one group the direction of monkey’s saccadic choice is congruent with the direction of his intrinsic bias, and another group is incongruent. After that, we plotted the average microsaccades direction for both data groups to show the continuous rotation of averaged microsaccades direction (left panels of Fig. 8A, 8B). At the same time, we applied a linear regression to microsaccade direction versus microsaccade generation time, and
then used the slope of the linear regression as the rotation speed of microsaccades direction. For both monkeys, the result shows that the rotation speed of microsaccades direction was significantly higher ($p < 0.001$ for monkey Datou and $p = 0.003$ for monkey Abao, permutation test) when the choice direction was incongruent with their intrinsically biased direction than when the choice direction was congruent with their intrinsically biased direction (right panels of Fig. 8A, 8B).

Since the saccadic target direction and saccadic choice direction are consistent in the correct trials, one argument is that the above results might be due to the congruency between the intrinsic directional bias of monkeys and the saccadic target location, rather than saccadic choice. To prevent this confusion, we analyzed the data of microsaccades in wrong trials (responsive saccades directed to the candidate goal on the opposite side of the visual cue in the SCIS task) from monkey Datou. The result shows that even when the monkey’s choice is wrong, the rotation speed is still higher when the chosen saccadic direction is incongruent with monkey’s intrinsic directional bias ($p = 0.041$, permutation test) compared with when the chosen saccadic direction is congruent with monkey’s intrinsic directional bias, which verifies that the rotation speed is not affected by the physical location of saccadic target but correlated with monkeys’ saccadic choice (Fig. 9). Since there were not enough wrong trials for monkey Abao, we were not able to do the same analysis for him. Nonetheless, the results from monkey Datou show, at least gives a strong hint, that the rotation speed of microsaccades direction is associated with monkeys’ saccadic choice rather than the physical location of saccadic target.

**Rotation of Microsaccades Direction Was Not Correlated with the Location of Visual Cue or Attention**

Finally, we checked the probability that rotation of microsaccades direction represents visual input or attention. If this was the case, then the rotation of microsaccades direction should differ for different spatial cue locations. For the number of trials from each monkey was limited, instead of dividing all correct trials from each monkey into 24 groups according to 24 different spatial cue locations, we divided them into 4 groups according to whether the spatial cue was presented in the upper or lower visual field and congruent or incongruent with the monkey’s biased direction. Then we analyzed the rotation speed of microsaccades direction for upper versus lower spatial cues and congruent versus incongruent spatial cues. It was found that, for both monkeys, the rotation speed of microsaccades direction was similar for upper and lower spatial cues (for monkey Datou, $p = 0.288$ for congruent condition and $p = 0.330$ for incongruent condition; for monkey Abao, $p = 0.459$ for congruent condition and $p = 0.409$ for incongruent condition), while it was significantly different for congruent and incongruent spatial cues (for monkey Datou, $p = 0.002$ for upper condition and $p = 0.001$ for lower condition; for monkey Abao, $p = 0.002$ for upper condition and $p = 0.010$ for lower condition).
for lower condition) (see Fig. 10A, 10B). Similar results were seen when separated trials to 4 conditions according to the location of visual cue in mediolateral dimension of left and right visual field (Fig. 10C, 10D).

This result indicates that the rotation of microsaccades direction is driven by neither visual cue nor covert attention induced by visual cues but monkeys’ saccadic choice.

**Conceptual Model for Microsaccades Direction Rotation**

Although the neuronal mechanisms cannot be specified, we devised a conceptual model (Fig. 11) to explain why the rotation speed of microsaccades direction is higher when monkeys choose a spatial saccadic target against their intrinsic directional bias compared with that when monkeys choose one consistent with their intrinsic directional bias. This model defines three brain states and associates transitions between brain states with different choices. The brain is in the “Initial State” before a monkey makes any choice, and it will transit to either “State A” or “State B” once the monkey chooses Choice A or Choice B respectively. In a condition without bias, transitions from the “Initial State” to “State A” and “State B” are equal in distance, thus they are of the same neutral speed with a fixed transition time corresponding to the fixed delay period in the SCIS task. Then we take into consideration the monkey’s inner bias which will shift the “Initial State” towards the brain state corresponding to the biased choice of the monkey and become the “Biased Initial State”. Suppose the monkey is biased to choice A, then the transition distance from the “Biased Initial State” to “State A” will be shorter than that from the “Biased Initial State” to “State B”; given that the transition time is fixed, the shorter transition distance from the “Biased Initial State” to the “State A” results in a lower-than-neutral transition speed, while the longer transition distance from the “Biased Initial State” to “State B” causes a higher-than-neutral transition speed. Now, we associate transitions between two brain states with the rotation of microsaccades direction, which leads to the observation that the rotation speed of microsaccades direction is higher when the monkey chooses against its intrinsic directional bias than that when the monkey chooses following its inner bias.

**Discussion**

Compared with previous studies, we investigated microsaccades in a very different way. For the first time in the literature studying microsaccades, we finely analyzed the spatiotemporal dynamics of microsaccades in a spatial cue instruct saccade task (SCIS), and found that microsaccades direction rotated during delay interval. Instead of visual cue induced attention modulation on microsaccades direction, the current study reveals
a correlation between rotation of microsaccades direction and the interaction of saccadic choice and intrinsic directional bias of monkeys, which is in support of complex roles of microsaccades in cognitive processes. The new perspective adopted in the current study raises questions about the dynamics of various modulations on microsaccades direction, e.g., does attention modulate microsaccade direction by solely increasing the proportion of microsaccades directed towards the attended location or by biasing microsaccade direction towards the attended location? In addition, the rotation phenomenon of microsaccades direction is also in line with a microsaccade-saccade continuum, namely, microsaccades and saccades play or are involved in similar functional roles. Currently, it is not easy to find a simple neuronal explanation for this rotation phenomenon of microsaccades direction, but there are some hints from neurophysiology studies about microsaccades generation within SC [39-43]; besides, this rotation phenomenon can be well addressed in a conceptual framework. Further studies can be either psychological or neurophysiological. Psychologically, typical decision making paradigms [44-46] can be applied to see whether the choice effect on rotation speed is coupled with the decision making process, or whether the rotation of microsaccades direction is caused by evidence accumulation [47, 48]; besides, it is also interesting to investigate the influence of different spatial arrangements of saccadic choices on the dynamics of microsaccades direction. At the neuronal level, it is worth to investigate the neuronal mechanisms underlying the rotation phenomenon of microsaccades rotation. Perhaps SC should be checked first to see whether there is a “moving hill” in the rostral pole when monkeys making a choice between two peripheral targets.

**A New Perspective towards Microsaccades Direction**

Our study focus on the elaborate dynamics of microsaccades direction along time, which provides a new perspective towards the study on microsaccades direction. Previous studies on microsaccade direction either focused on discrete time points hence a lack of fine temporal resolution [24, 25, 28] or reduced the spatial resolution by selecting microsaccades towards large direction ranges [27, 29] or even discarding vertical (or horizontal) components of microsaccades [23, 25, 26, 30]. Although these studies revealed important characteristics of microsaccades, e.g., microsaccades direction can be modulated by covert attention, questions involving elaborate dynamics of microsaccades direction along time could not be answered by such studies because of limitations of perspectives taken in them. Here we give three example questions which are fundamental regarding mechanisms underlying attention modulation on microsaccades direction: 1). Is attention modulation a transient effect which affects microsaccades direction shortly after attention shifts and then diminishes, or a “ramping” and persistent effect as long as attention holds? 2). How long does it take for attention modulation on microsaccades direction to take effect? 3). Does attention modulate microsaccades direction by attracting the whole direction distribution towards the attended location or
merely increasing fraction of microsaccades towards the attended location? To answer these important question, one needs to not only finely direct attention but also carefully look into the dynamics of microsaccades direction in both temporal and spatial dimension.

**Microsaccade as a Visual-Oculomotor Complex**

A large body of literatures suggests microsaccade as a visual-oculomotor complex. First of all, microsaccades are intensively involved in visual-oculomotor processes. Microsaccades were reported to prevent visual fading [15, 16], assist with visual sampling near the fovea [17-22], reveal visual contrast sensitivity [49], induce microsaccadic suppression [50-55] and microsaccadic compression [55, 56], and influence [27, 52, 53, 57, 58] or be modulated by [59] visually guided behaviors. There are also bunches of neuronal evidences indicating complex involvements of microsaccades in various brain areas within the visual-oculomotor pathway, including lateral geniculate nucleus (LGN) [60], primary visual cortex (V1) [60-64], extrastriate visual cortex (V2 and V4) [61], middle temporal visual area (MT) [65], superior colliculus (SC) [39, 66] and frontal eye fields (FEF) [66].

Moreover, being associated with various known and unknown factors, microsaccades direction implicates even more complicated ways in which microsaccades participate in cognitive processes. Microsaccades direction could be directed towards visual information near the fovea [18, 20], reflect saccadic choice and the corresponding reward size [30], be different for binocular and monocular microsaccades [67], and be modulated by the allocation of visuospatial attention [23-29]. In addition, the debate on how reliably microsaccades direction indicates covert attention [68-71] strengthens the notion of complex cognitive involvements of microsaccades.

Idiosyncrasies of microsaccades direction is another obscure propensity of microsaccades: it was very common to observe additional components in the distribution of microsaccades which could be hardly explained by experimental settings [24] [28] [72]. Factors such as visual information, visuospatial attention and saccadic choice mentioned above can only modulate microsaccades direction but not determine the whole distribution of microsaccades direction. Therefore, there should be presumably many more unknown factors, either endogenous or exogenous, influencing microsaccades direction.

Besides, microsaccades rate was reported to be associated with interactions between target location and reward size [72], difficulty of a nonvisual mental arithmetic task [73], arousal level [74] and even heartbeats [75].

Four aspects in the current study are in line with microsaccades as a visual-oculomotor complex.

1. No attention modulation on microsaccades was observed. The absence of attention
modulation might be due to a very brief allocation of attention to the spatial cue as in other behavioral tasks [23]. It is possible that monkeys shift their attention away from the spatial cue before attention could exert any effect on microsaccades direction, especially when saccadic choice is affecting microsaccades direction. Because attention allocation was not carefully controlled in our study, it is not necessary for our results to contradict with the notion that attention can modulate microsaccades direction. However, our results do indicate that, to observe attention modulation on microsaccades direction, one should at least carefully control attention allocation and exclude possible overwhelming of attention effect by other factors such as saccadic choice.

2. Distributions of microsaccades direction are idiosyncratic for both monkeys. For our two monkeys, microsaccades during the delay period were mostly upward and downward respectively (See Fig. 5), similar to those observed in [24, 72]. One possibility causing the idiosyncrasies of microsaccades direction in our monkeys might be that monkeys made their efforts to avoid break fixations towards the two candidate goals.

3. Not microsaccades direction but the changing process of microsaccades direction was modulated by monkeys' saccadic choice. Very recently, microsaccades direction was found to be modulated by saccadic choice and the strength of the modulation was positively correlated with the subjective value of the saccadic choice [30]. However, this kind of direct correlation between microsaccades direction per se and saccadic choice was absent in our study, and instead, we found a correlation between the change of microsaccades direction and saccadic choice. A possible explanation for this was that the idiosyncrasy of microsaccades direction presented in our study intervened with the possible correlation between microsaccades direction and saccadic choice, since the directional distributions of microsaccades were very different in our study compared with those in the previous study which well followed saccadic directions. Above all, our study suggests more complex cognitive roles of microsaccades than implications from previous studies, and further raises a series of critical questions like what is the dynamic process of attention modulation on microsaccades direction along time. To solve such questions, one should apply the new perspective, namely, analyze detailed dynamics of microsaccades direction along time, as mentioned in the last section.

4. The rotation direction of microsaccades direction remains a puzzle. Because of this, our conceptual model does not involve any microsaccades direction information, although it perfectly explains the correlation between rotation speed of microsaccades direction and monkeys’ choice against their inner bias. However, there do exist some hints on possible explanations at the neuronal level of the SC [39, 41, 43], assuming that enhanced activity in caudal SC will attract activities in rostral SC and thus make microsaccades direction rotate. The rotation direction observed in monkey Datou is relatively easy to explain: two candidate goals evoke visual responses in caudal poles of both left and right SC [76], and the response in the left SC is enhanced due to the inner bias of Datou; then a leftward saccadic choice will only enhance the left response very little while a rightward one will largely enhance the right response [77], therefore little
rotation will happen for a leftward choice but apparent rotation towards right will be observed for a rightward choice. However, this explanation does not hold for monkey Abao, since the rotation direction for a leftward choice was rightward; it seems that there was an extra control signal from higher level projected on SC which altered the rotation direction – perhaps a signal generated by the monkey to avoid leftward break fixations for leftward choices.

**A Microsaccade-Saccade Continuum**

There is an evolving notion that microsaccades and saccades play similar functional roles, as a microsaccade-saccade continuum [17, 35, 41]. Microsaccades enhance visual processing near the fovea [18-22] while large saccades gather visual information by directing the high-acuity fovea to locations of interest. Similar to saccadic compression [78, 79] and suppression [50, 55, 80-86], microsaccades are also subjected to microsaccadic compression [55, 56] and microsaccadic suppression [50-55]. Besides, microsaccades and saccades share a common oculomotor generator [17, 39, 41, 57, 87, 88]. Further, attention modulation on microsaccades direction can be explained based on a continuum of microsaccades and saccades generation within the SC [39, 43]. In addition, both microsaccade [30, 72] and saccade [89, 90] can be affected by reward.

In consistence with this microsaccade-saccade continuum, the current study shows that the rotation of microsaccades direction is associated with monkeys’ saccadic choice against their inner bias, which is in line of saccades’ function of overtly selecting a saccadic target of interest. Further, the possible neuronal mechanism for the rotation direction of microsaccades direction discussed in the last section reveals that microsaccades and saccades share a generation continuum in the SC [39, 41].

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9. Sharpe, C., The visibility and fading of thin lines visualized by their controlled movement


FIG. 1: Paradigms of two tasks. For both tasks, the red dot in the center is the fixation point, and the gray arrows illustrate required response saccades. A, SCIS task. The two symmetric red dots on each side are the candidate goals. Green dots and dashed green circles illustrate 24 possible cue locations which were separated into two spatial categories (left and right). The monkeys needed to choose the target on the same side of the visual field as the cue and then make a response saccade towards it. B, MGS task. The red dot on the right is the target for this example trial, while dashed red circles are other 7 possible target locations.

FIG. 2: Density distribution of main sequence. Each dot represents a two-dimensional (amplitude versus peak velocity) bin and the color values the number of saccades in that bin. Red part is fixational saccades, while green part represents response saccades and fixational break saccades. A, B, monkey Datou. C, D, monkey Abao. A, C, SCIS task. B, D, MGS task.
FIG. 3: Microsaccades rate across all correct trials in the SCIS task and correct trials for horizontal target locations in the MGS task. The dashed lines represent the time of cue onset in the SCIS task (A, C) or target onset in the MGS task (B, D). A, B, monkey Datou. C, D, monkey Abao. Note that, during the testing period, only the first microsaccade for each trial was used.
From 129 ms after target onset
From 115 ms after cue onset
From 152 ms after target onset
From 136 ms after cue onset

FIG. 4: Density distribution of directions of all pairs of consecutive saccades during the testing period for all correct trials. The horizontal axis represents the direction of the first saccade in a pair while vertical axis the direction of the second saccade in that pair. The color of each bin means the number of pairs of saccades falling in that bin. Dashed lines mean that the second saccade is exactly opposite in direction to the first saccade. A, B, monkey Datou. C, D, monkey Abao. A, C, SCIS task. B, D, MGS task.

FIG. 5: Direction distribution of first microsaccades during the testing period for all correct trials in the SCIS task. A, B, monkey Datou. C, D, monkey Abao. A, C, target on the left. B, D, target on the right.
FIG. 6: Distribution of microsaccades direction along time for correct trials. Horizontal axis represents time and vertical microsaccades direction (0 for rightward), and each dot represents a two-dimensional (direction vs. time) bin of 2 and 10 ms, and its color indicates the number of microsaccades (normalized for each monkey in each task based on the maximum number) in it. A, C, SCIS task. B, D, MGS task. A, B, monkey Datou. C, D, monkey Abao. For each subfigure, the left panel illustrates the case when the target was presented on the left, while the right panel the condition when target was on the right. Green dashed lines represent time of cue onset in the SCIS task or target onset in the MGS task. Blue lines represent linear fittings for microsaccades direction versus time during the testing period for the SCIS task. Note that for each panel, during the testing period, only first microsaccades were included; and for the SCIS task, only microsaccades towards the up visual hemi field for monkey Datou and only microsaccades towards the down visual hemi field for monkey Abao were used for linear fittings (see Materials and Methods).
FIG. 7: Direction bias of break saccades of both monkeys performing the SCIS task. A, C, break saccades during the period from fixation on to candidates on. B, D, break saccades during the period from candidates on to cue on. A, B, monkey Datou. C, D, monkey Abao. For each subfigure, the left panel shows the proportions of break saccades towards left versus those towards right, and each triangle represents data from one month; the right panel illustrates average proportions of leftward and rightward break saccades across all months with SEM. Paired Wilcoxon signed rank test (single-tailed) was used for significance analysis.

FIG. 8: Rotation of microsaccades direction during the testing period for all correct trials under different saccadic choices for both monkeys performing the SCIS task, congruent means monkeys choice is consistent with the biased direction while incongruent means the opposite condition. A, monkey Datou. B, monkey Abao. For each subfigure, the left panel illustrates average dynamics of microsaccades direction during the testing period, while the right panel shows the average rotation speed of microsaccades direction for different spatial cue conditions, each number above or beneath two bars is the p-value out of a permutation test. Shaded areas and error bars represent SEM.
FIG. 9: Similar illustrations with Fig. 8, but with all wrong trials from monkey Datou performing the SCIS task.

FIG. 10: Similar illustrations with Fig. 8, but both the congruent and incongruent correct trials for both monkeys were divided into two subgroups according to the location of the spatial cue: A, B, upper (vertical locations of 5° and 10°) versus lower (vertical locations of −5° and −10°); C, D, middle (horizontal locations of ±4°) versus lateral (horizontal locations of ±12°).
FIG. 11: Conceptual model which explains how bias influences the rotation speed of microsaccades direction when monkeys make different choices. Initial State represents monkeys brain state before choice, State A and B indicate two different brain states corresponding to monkeys Choice A and Choice B respectively. Based on the fixed interval from cue on to a response saccade allowed (600ms, see SCIS task in Fig. 1), a monkey without any direction bias would transit its brain state from Initial State to State A or State B with the same neutral speed; while for a monkey with a bias, say a leftward bias, the Initial State will shift to the Biased Initial State which is closer to State A, thus the transition to State A will be slower than that to State B, considering the same transition time.