Tracking Real Time Changes in Working Memory Updating and Gating with Event-Based Eye-Blink Rate

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Abstract

Effective working memory (WM) functioning depends on the gating process which regulates between maintenance and updating in WM. The present study used event-based eye-blink rate (ebEBR), which presumably reflects phasic striatal dopamine activity, to examine how the cognitive processes of gating and updating separately facilitate flexible updating of WM contents and the potential involvement of dopamine in these processes. Real-time changes in eye-blinks were tracked during performance on the reference-back task, in which demands on these two processes are independently manipulated. In all three experiments, trials which required WM updating, and trials, which required gate switching, were both associated with increased ebEBR. These results may support the *prefrontal cortex basal ganglia WM model* (PBWM) by linking updating and gating to striatal dopaminergic activity. In Experiment 3, ebEBR was used to determine what triggers gate switching. We found that switching to an updating mode (gate opening) is more stimulus driven and retroactive than switching to a maintenance mode, which is more context driven. Together, these findings show that ebEBR – a cheap, non-invasive, easy-to-use measure – can be used to track changes in WM demands during task performance, and hence, possibly of striatal dopamine activity.

Introduction

Working memory (WM) is a dynamic system in which goal-relevant information is temporarily maintained and manipulated. Effective WM functioning depends on filtering or 'gating' mechanisms that "decides" which information is relevant, and hence should enter WM, and which is not, and should be left outside. Optimizing the balance between these two requirements is described in the literature as the stability-flexibility dilemma [1-3]. WM faces two seemingly opposing demands. First, the maintained information within WM must be shielded from being overridden by task-irrelevant input. Due to its capacity limit [4], storage of irrelevant information in WM would necessarily be at the expense of the ability to maintain relevant contents. At the same time, task-relevant information must be recognized and entered into WM, in order to accommodate to relevant changes in the environment. Therefore, encoding information to WM must be selective[5-8].

The prefrontal cortex basal ganglia WM model (PBWM)[9,10] (for alternative models see [11,12]) is a physiologically based computational model that describes the mechanisms by which maintenance and updating are coordinated. According to PBWM, the prefrontal cortex (PFC) is responsible for robust, active maintenance of information in a distractor-resistant manner, achieved through recurrent excitation [10,11,13-17]. Controlling the flow of information into WM is done by the basal ganglia (BG), which serve as a "gate" to WM [6, 18]. When closed, the gate enables active maintenance and stability within WM by preventing irrelevant input from entering WM. However, when new input is relevant, the gate opens and enables flexible updating of the existing representations. Control over the state of the gate (namely, open or closed) is done through dopaminergic (DA) activity [9, 11, 20,21]. The PBWM model assumes that the gate to WM is closed by default, and hence each instance of updating should be followed by gate closing.

In line with the PBWM model, a large body of work shows that DA serves an important role both in supporting maintenance and updating of WM and in their coordination. Stability of representation in WM is managed by tonic DA activity in the PFC, while phasic DA from dorsal striatum drives the gate-opening signal that leads to the updating of WM by disinhibiting the thalamus. However, the effectiveness of the phasic DA in overriding tonic DA depends on the initial striatal tonic level [12,22,23]. The disinhibition of the thalamus, gates information into the PFC, and thereby flexibly updates WM. This updating is also marked by the phasic DA signal [10,24].

A growing body of research indicates that spontaneous eye-blinks (sEB) may be an effective measure of striatal DA activity. sEB are endogenous and unconscious responses [25], which occur in the absence of any evident stimulus. Although the neural mechanisms that underlie sEB are not yet fully understood, converging evidence from clinical and pharmacological studies indicate a positive correlation between striatal DA activity and the rate of eye-blinks (i.e. the number of sEB per minute) (sEBR) in resting state [26-30]. For example, two disorders characterized by DA dysfunction, Parkinson and Schizophrenia, are associated with decreased [28,29] and increased [27,32] sEBR, respectively. Furthermore, DA agonists increase sEBR, while DA antagonists decrease sEBR[30,33-35]. Notably, sEBR in resting state conditions is correlated with subsequent performance on cognitive tasks that are known to depend on DA neurotransmission, including the stop-signal task[36], attentional blink[37], attentional bias[38], and task switching[39]. As these associations were found using resting-state sEBR, they suggest a relationship between tonic DA activity and cognitive functioning. Moreover, sEBR has been related to avoidance learning and not to positive learning, which may suggest that sEBR specifically reflects the activity of the DA D2 receptor[40]. This idea is supported by a recent PET study in monkeys, which found a strong correlation between sEBR and D2-like receptor availability in the ventral striatum and caudate

nucleus[41]. Furthermore, in this study, D2-like receptor availability correlated with D2-like receptor agonist-induced changes in sEBR and the density of D2-like receptors determined in vitro. Thus, convergent evidence from different lines of research indicates that striatal DA activity regulates sEBR.

sEBR provide an easy-to-obtain, non-invasive and cheap method for assessing the relationship between striatal DA function and behavior without the need to alter the natural DA activity in the brain. However, previous work has left unresolved to what extent sEBR can be used to track real-time changes in DA activity *during* task performance, as a function of task demands. Task evoked eye blinks is a relatively new method[42-49] compared to the resting state method. To our knowledge, only two studies have so far used ebEBR in order to test the involvement of fronto-striatal DA in WM updating, both in infants. The first was done during an incidental hierarchical rule learning task in 8 month old infants[49]. The authors found increased ebEBR when the task rule was updated in WM compare to when it was repeated. The second was done during an A not B WM task in 10 month old infants[42]. The authors found that ebEBR was increased when the location of the hidden toy had to be updated in WM compared to when the location of the toy was revealed. These initial findings indicate that ebEBR is dynamically modulated by WM processes known to depend on DA activity, although it is unclear to what extent these findings extent to the adult brain.

The aim of the current study was to shed light on the involvement of DA in gating and WM updating, by examining task demand-related changes in eye-blink rate during performance on the reference back task[50,51]. The reference-back is a novel paradigm which allows separation of processes related to working memory updating from processes related to gate opening and closing. This task is composed of two types of trials, *reference* and *comparison*, which are indicated by different colors (e.g., a red or blue frame surrounding the stimulus, respectively; see Figure 1). In each trial, participants are required to indicate whether

the presented item is the same as, or different from, the most recent item that appeared within a red frame. Accordingly, each trial in this task requires a comparison to the reference followed by a same/different decision. In addition, reference trials require one to update WM with the presented stimulus, because it should serve as a reference to which the following trials should be compared. Thus, reference trials require opening the gate to WM, in order to enable updating. On the other hand, comparison trials do not require WM updating. Instead, these trials require one to continue maintaining the last reference stimulus in WM. Because each comparison trial is also compared to the last reference trial, the reference needs to be protected from being overwritten by changes in comparison trials. Hence, the gate over WM should be closed in these trials. Pervious results using this paradigm[50,51] demonstrate that (a) performance in reference trials is slower than in comparison trials, supporting the additional updating process required in the former, and (b) switching between the two trial types is associated with an additional cost, reflecting the time taken to open or close the gate to WM[52,53].

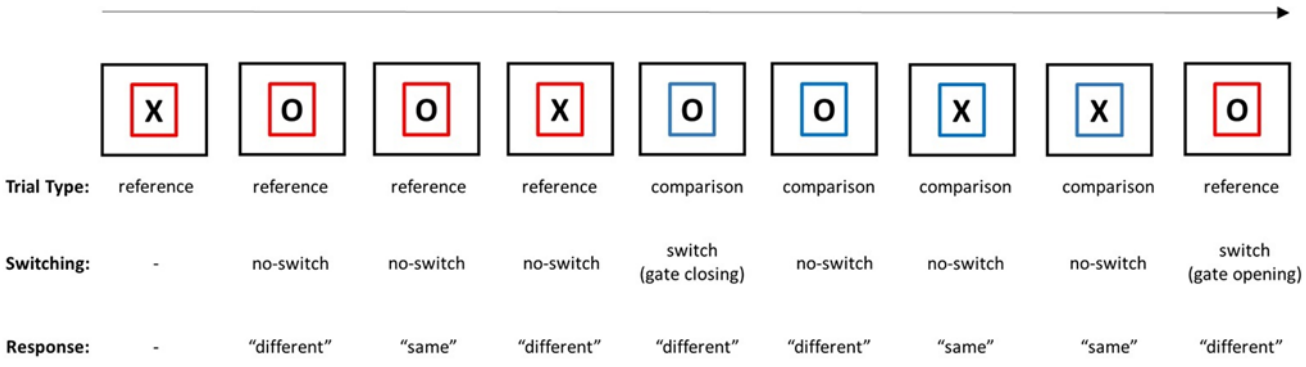

Figure 1 | The reference-back task. Trials with a red frame are reference trials and trials with a blue frame are comparison trials (see text for details). The sequence length for each trial-type was constant of 4 trials. There was a fixation display after response for 2s thus creating a 2s inter trial interval (ITI). The state of the gate and the correct response for each trial are indicted below each stimulus display.

In three experiments, we intended to determine if ebEBR can be used to track changes in demands on gating and updating. Inspired by the PBWM model and our previous results[50,51] and under the assumption that ebEBR reflects phasic DA activity, we predicted that WM updating and gate opening would be associated with an increase in ebEBR. Gate closing would not be accompanied by an increase in ebEBR because, as implied in the model, gate closing is the default state of the gate and does not require phasic DA. In Experiment 1, we tested this main prediction. In Experiment 2, we aimed to replicate the findings of Experiment 1 and extend these by examine the optimal window size in which event related ebEBR is sensitive to updating and gating. Finally, in Experiment 3, we investigated to what extent observed changes in ebEBR were stimulus dependent. Specifically, we aimed to determine if when we allow certain cognitive processes to be active *before* stimulus presentation, the ebEBR effect will also shift in time to the pre-stimulus interval? (see Figure 5) If so, it may help understand to what extent gating is stimulus-driven. Stimulus driven gating is a retroactive strategy where all the information on the input is required in order to make a decision to switch state or not. Alternatively, context driven gating, would result from a more proactive strategy, where the stimulus is not crucial element of the decision, but rather, the context is enough. In the reference back task, the context is all the information on the trial-type, i.e., if it is a reference or comparison trial, if it is a switch or a no-switch trial and the history of the trials.

Results

Experiment 1

Our main prediction was that changes in WM task demands, specifically gate opening and updating, would be associated with changes in ebEBR. A 2-way ANOVA was conducted on the ebEBR data with, Trial-Type (reference, comparison) and Switching (switch, no-switch) as within-subject independent variables (see Figure 2). Indeed, an increased ebEBR was observed in reference than in comparison trials, *F(1,18)=13.63, MSe=.0013, p=.002,* η_p^2*=.43.* ebEBR was also increased in switch compared to no-switch trials, *F(1,18)=24.48, MSe=.0015, p<.001,* η_p^2*=.58.* The two-way interaction was non-significant, *F(1,18)=.37, MSe=.0010, p=.55,* η_p^2*=.02.*

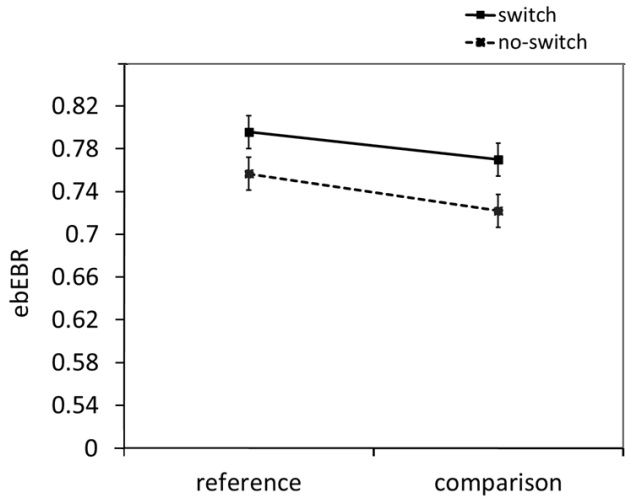

Figure 2 | The ebEBR results of Experiment 1. Error bars represent 95% confidence intervals[59].

Experiment 2

In order to test the optimal window size in which ebEBR is sensitive to task demands, we divided the stimulus- locked 4sec window into two halves of 2sec each. A 3-way ANOVA

was conducted on the ebEBR data with Segment Part (first half, second half), Trial-Type (reference, comparison) and Switching (switch, no-switch) as within-subject independent variables (see Figure 3). As before, the main effects of Trial-Type *F(1,19)=15.37, MSe=.0015, p<.001,* η_p^2*=.45* and Switching, *F(1,19)=11.41, MSe=.0015, p=.003,* η_p^2*=.37* were significant. The two-way interaction between Switching and Segment Part was also significant, *F(1,19)=4.91, MSe=.0015, p=.04,* η_p^2*=.47* (see Figure 3). Specifically, the simple main effect of Switching was significant in the second time window, *F(1,19)=16.57, MSe=.0015, p<.001,* η_p^2*=.20*, but not in the first, *F(1,19)=.62, MSe=.0016, p=.44,* η_p^2*=.03*. Thus, the increase in ebEBR in switch compare to no-switch trials was more pronounced in the second compared to the first half of the trial. At this stage, the reasons for the delayed effect of switching in Experiment 2, compared to Experiment 1, are not fully understood. One possibility is that the prolonged ITI in Experiment 2 led to adopting a more retroactive strategy, in which updating and possibly gating, were carried out after the response was indicated. The two-way interaction between Segment Part and Trial-Type was marginally significant, *F(1,19)=4.20, MSe=.0013, p=.05,* η_p^2*=.18.* The three-way interaction was non-significant, *F(1,19)=1.20, MSe=.0012, P=0.29,* η_p^2*=.06.*

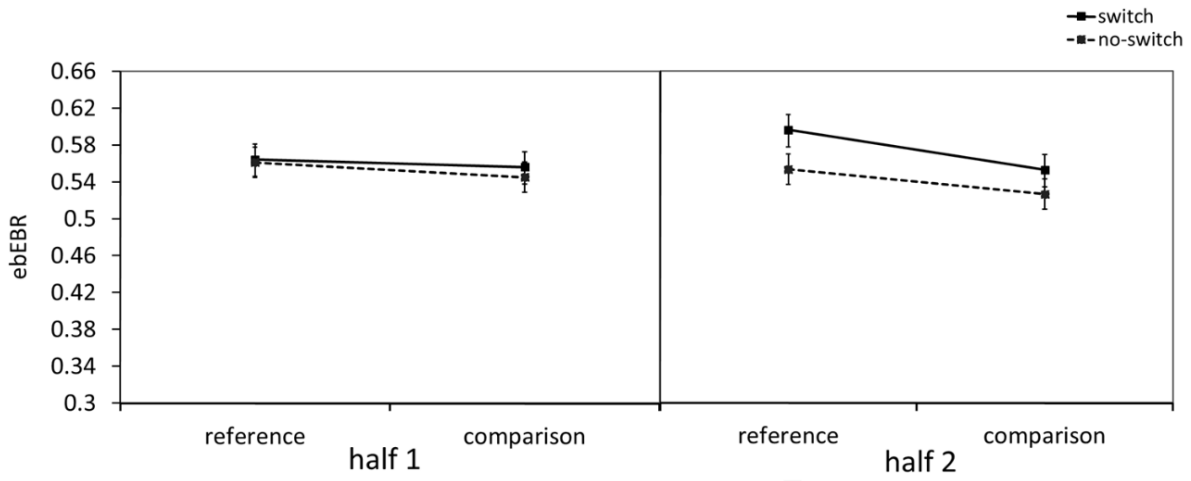

Figure 3 | The ebEBR results of Experiment 2. The 4sec segment length was divided to two parts, 2sec each. ebEBR as a function of Segment part, Trial-Type and Switching is

presented. The effect of switching is observed only in the second half of the segment. Error bars represent 95% confidence intervals.

Experiment 3

In order to test what triggers the gate, a "random version" of the reference back task was used, in which both trial types could appear in each trial with equal probabilities (see Figure 5). Crucially, each trial began with a 4sec cue, which indicated the upcoming trial type before the stimulus was presented, but did not provide explicit information about the stimulus identity. Thus, in the cue phase participants knew if this would be a reference or a comparison trial, which also implies whether this trial would be a switch or a no switch trial. However, as they had no information about stimulus identity at this point, they could not make response-related decisions and could not prepare a motor response. If a switch cost in ebEBR will be observed in the cue phase it will suggest that the processes involved in switch trials (presumably gate opening and closing) do not require the stimulus. However, if switch cost will be detected in ebEBR only after the stimulus was presented it would support the stimulus-driven hypothesis. Stimulus driven gating is a retroactive strategy where all the information on the input is required in order to make a decision to switch state or not. Alternatively, context driven gating, would result from a more proactive strategy, where the stimulus is not crucial element of the decision, but rather, the context is enough. In the reference back task, the context is all the information on the trial-type, i.e., if it is a reference or comparison trial, if it is a switch or a no-switch trial and the history of the trials.

Cue locked analysis

A 3-way ANOVA was conducted on the ebEBR data with Segment Part (first half, second half), Trial-Type (reference, comparison) and Switching (switch, no-switch) as within-subject independent variables (see Figure 4a). The effect of Segment Part was significant, $F(1,20)=79.74$, $MSe=.0129$, $p<.001$, $\eta_p^2=.80$ and so was the two-way interaction between Segment Part and Switching, $F(1,20)=5.05$, $MSe=.0019$, $p=.04$, $\eta_p^2=.20$. This interaction effect indicated a larger switch cost in the first half of the segment than in the second half (0.03 vs. -0.003 ebEBR per second, respectively). The two-way interaction between Switching and Trial-Type was also significant, $F(1,20)=11.89$, $MSe=.0020$, $p=.002$, $\eta_p^2=.37$, reflecting increased ebEBR on switch trials compare to no-switch trials only in comparison trials, $F(1,20)=13.72$, $MSe=.002$, $p=.001$, $\eta_p^2=.41$, but not in reference trials, $F(1,20)=1.90$, $MSe=.0016$, $p=.18$, $\eta_p^2=.09$. None of the other effects were significant, all Fs<3.61.

Stimulus locked analysis

The effect of stimulus presentation on ebEBR was examined in a 3-way ANOVA with Segment Part (first half, second half), Trial-Type (reference, comparison) and Switching (switch, no-switch) as within-subject independent variables (Figure 4b). The ebEBR pattern did not differ between the two segment parts. Neither the main effect of Segment Part nor any interaction that includes this factor were significant, all Fs<1. The main effects of Trial-Type, $F(1,20)=11.50$, $MSe=.0021$, $p=.003$, $\eta_p^2=.36$, was significant, reflecting a higher ebEBR in reference trials than in comparison trials. Also, ebEBR was larger in switch trials than in non-switch trials, $F(1,20)=5.75$, $MSe=.0032$, $p=.03$, $\eta_p^2=.22$ as in the original analysis. The two-way interaction between Trial-Type and Switching was only marginally significant, $F(1,20)=3.91$, $MSe=.0012$, $p=.06$, $\eta_p^2=.16$.

Because the cue locked analysis revealed a significant switch cost only in comparison trials and not in reference trials we decided to decompose the two-way interaction of Trial-Type and Switching to its simple effects. This analysis showed a mirror image of the two-way interaction in the cue locked analysis. A significant switch cost was only observed in reference trials, $F(1,20)=11.96$, $MSe=.001$, $p=.002$, $\eta_p^2=.37$ but not in comparison trials, $F(1,20)=.74$, $MSe=.001$, $p=.40$, $\eta_p^2=.04$.

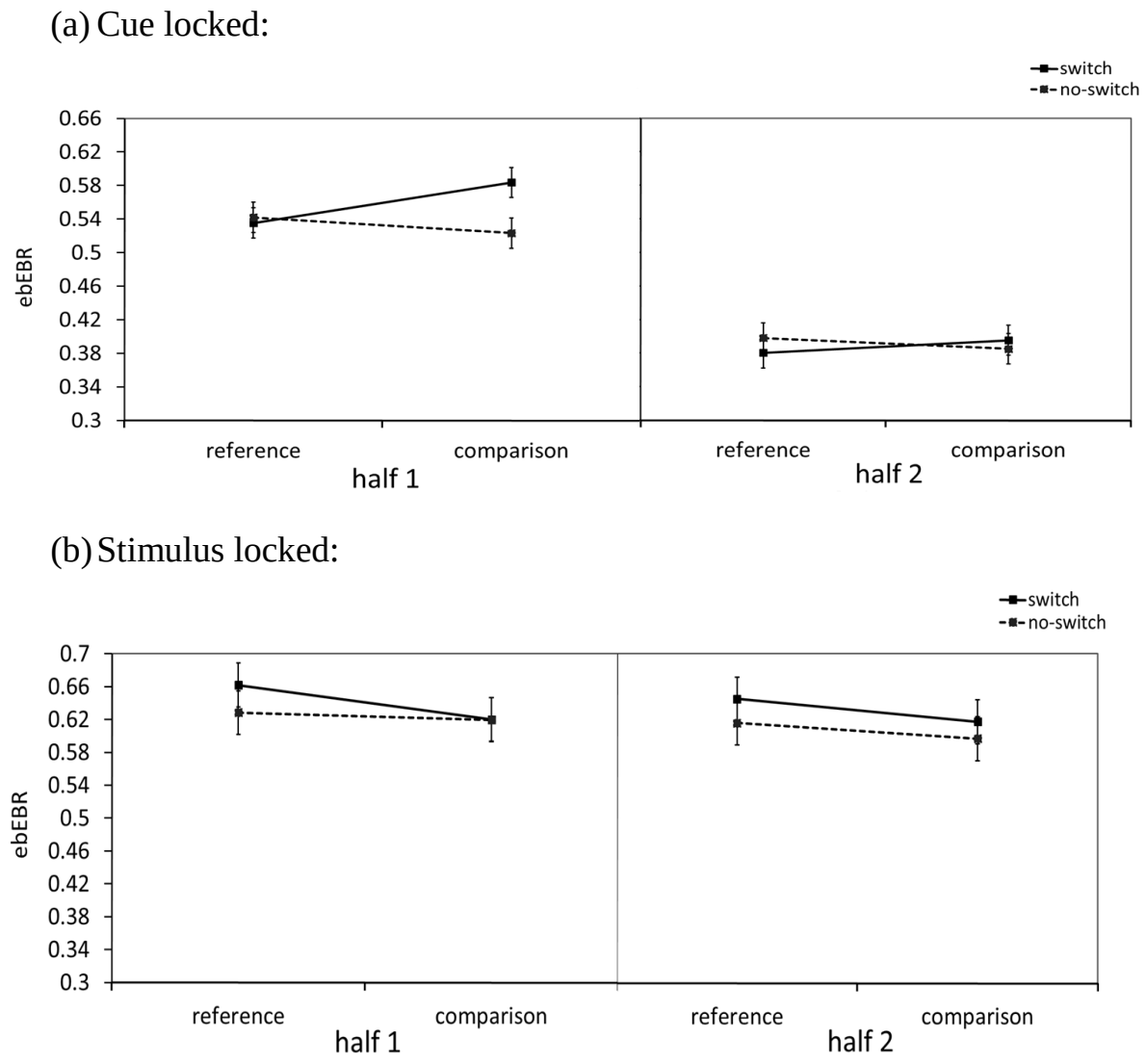

Figure 4 | The ebEBR results of Experiment 3. ebEBR as a function of Segment part, Trial-Type and Switching is presented. The 4sec segment length after (a) cue presentation and after (b) stimulus presentation were divided to two parts, each of 2sec. In the cue-locked presentation, the switch cost is observed only in comparison trials and only in the first half of

the segment. In the stimulus-locked presentation, the switch cost in reference trials is observed in both segment parts. Error bars represent 95% confidence intervals.

Discussion

In this study, we demonstrated that ebEBR, a cheap, non-invasive and easy-to-use measure, which presumably reflects striatal dopamine activity [26,30,40,49], can be used to track changes in demands on WM during task performance. The ebEBR results in all three experiments demonstrated that ebEBR follows changes in WM demands with a resolution of a few seconds, thereby extending previous studies that reported a relationship between performance on cognitive tasks and the more familiar sEBR measure, which is recorded over several minutes during resting conditions[37-39]. Specifically, ebEBR recorded over 4 and even 2sec increased in conditions of the reference-back, which presumably rely on fronto-striatal DA[9], and mirrored the behavioral results (see supplementary for behavioral results). Specifically, reference trials, which required updating of WM and trials which required switching the state of the gate, lead to an increase in ebEBR, in line with our predictions which were inspired by the PBWM model[9,10] with the exception that the reported results suggest that gate closing might also be DA-dependent. The reported findings may suggest that gate closing is not automatic but rather like gate opening might involve phasic DA response, and that perhaps the default state of the gate is not always closed but rather might be dependent on context[51]. More generally, these findings suggest that the ebEBR method[42,49] is a viable method to track DA based changes in WM demands during task performance.

Finally, in Experiment 3, the on-line measure of ebEBR enabled testing whether contextual information provided by a cue can be used to prepare in advance for gate switching. The ebEBR analysis revealed a dissociation in preparation time between switching

to reference (updating mode) and switching to comparison (maintenance mode). Specifically, the context cue lead to an increase in ebEBR before the stimulus was presented only when switching to comparison. When switching to reference, this increase in ebEBR was, however, observed only after the stimulus was presented. These novel findings may suggest that gate closing is more proactive than gate opening. This is perhaps because updating of WM is initiated only when the stimulus is changed. Thus, contextual information (in the form of a red frame) only provides the information that updating is possible, which may not be enough to trigger gate opening. In contrast, switching to a maintenance mode can be initiated by contextual information (in the form of a blue frame) because it provides the information that no updating will be required and thus may trigger gate closing without the information of stimulus. The process of gate opening after stimulus presentation may nevertheless be facilitated by knowledge of an upcoming switch, as in Experiment 3, the gate opening-related increase in ebEBR was already observed in the first 2sec after stimulus onset, while in Experiment 2 the switching effect was detectable only in the last 2sec of the segment. Evidence for preparation is also indicated by the significantly reduced switch cost in RT compared to Experiments 1 and 2 (see Supplementary for behavioral results). Indeed, studies have shown that the cognitive system can at least partially be reconfigured in preparation for a switch in task set[54,55].

To conclude, together, our findings from three experiments confirm that ebEBR can be used as a measure of cognitive control over WM; The significant switching cost observed in the cue phase analysis illustrates that ebEBR can dynamically track cognitive control processes which are not tied to any response-related processes, and thereby provide information that cannot be extracted from RT patterns alone. More generally, the reported ebEBR results provide further support for the notion that ebEBR can be used to track changes of cognitive functions which are based on striatal DA activity, during task performance.

We acknowledge that while EBR is an established physiological indicator of striatal DA activity[26-30], EBR is still an indirect measure of striatal DA activity. Future studies, that combine ebEBR measurements during task performance with pharmacological manipulations and pupil measurement and/or neuroimaging, are necessary to establish the neurochemical and neural mechanisms underlying the observed ebEBR modulations. For example, it would help determine to what extent the event-based EB effects that we found here are related to WM processes and phasic DA activity vs. non-specific processes, such as arousal[45,56]. Nevertheless, our findings indicate that ebEBR provides a viable online measure that can aid in investigating DA based cognitive processes also in populations in which pharmacological alteration of DA is not feasible or sensible, such as in infants, elderly and recreational cocaine users. As such, this method may enhance our understanding of the mechanisms underlying cognitive dysfunction in psychiatric disorders characterized by DA abnormalities, such as Parkinson, Schizophrenia and ADHD.

Method

Participants

20 undergraduate students from Ben-Gurion University of the Negev participated in Experiment 1 (6 males; age: M = 24.45, S.D. = 2.06) and in Experiment 2 (3 males; age: M = 24.15, S.D. = .91). 22 undergraduate students from Ben-Gurion University of the Negev participated in Experiment 3 (4 males; age: M = 22.81, S.D. = 1.43). One participant was removed from the analysis of Experiment 1 due to low accuracy in the reference back task (<50% in some of the conditions) and one participant was removed from the analysis of Experiment 3 due to noisy recording of the eye-blinks. Participants were either paid for their participation or received partial fulfillment of course requirements. Informed consent was

obtained from all participants in accordance with the guidelines of the Ethics Committee of the Psychology Department at Ben-Gurion University of the Negev who also approved the study.

Stimuli and Apparatus

Stimuli presentation and behavioral data collection were done using E-Prime v2.0 (Psychology Software Tools, Pittsburgh, PA). The stimuli were the letters “X” and “O”, in font size 36, presented in black against a light gray background within a red or a blue frame. Responses were collected using a serial response box. Note that a stimulus-set of only 2 stimuli was chosen for two reasons. First, it maximizes the control required in order to answer correctly. The smaller the stimulus set, the larger the probability that the present stimulus was presented in the previous trials, leading to a strong familiarity signal in each trial. Control is required in order to overcome familiarity and base the response on recollection, which addresses the precise context of the stimulus in WM. The most extreme case is with only 2 stimuli. Second, this design facilitates a balanced manipulation of stimuli and conditions as "same" and "different" responses are equally probable and thus so are the conditions preceding the response (match/no-update, mismatch/update).

Procedure

Each trial started with a presentation of the stimulus “X” or “O” at the center of the screen (see below the exception in Experiment 3). The stimulus was presented in black inside either a red or a blue frame. After the response, a fixation screen was presented with three dots at the center of the display to maintain foveal perception of participants. The reference-back task was comprised of two trial types, reference and comparison (see Figure 1). The stimulus in each trial (an X or O) was selected at random. The first trial in a block was always

presented in the reference color and did not require a response. In each of the following trials, the participants had to indicate whether the stimulus was the same as or different than the *most recent reference trial*. "Same" and "different" responses were indicated using the right and left index fingers, respectively, using a serial response box. Participants were instructed to be as fast as possible.

In Experiments 1 and 2 we used a fixed alternating-runs order of trial types, composed of 4 trials of each condition in a row (see Figure 1). In Experiment 3, the sequence length of each trial-type (reference, comparison) was random. The probability of a switch between trial types was 50% in each trial. A cue indicating the trial-type was added before stimulus presentation. Each trial was initiated with a cue, namely a red or blue empty frame, presented for 4sec (see Figure 5). In Experiment 1, the stimulus was presented until response. After the response, a fixation screen was presented for 2sec inter-trial interval (ITI). In Experiments 2 and 3, the stimulus was presented inside the frame until a response was given or until 3sec had elapsed. After the response, a fixation screen was presented until 4.5sec had elapsed from the stimulus presentation (i.e., the ITI was 4.5sec minus the RT in each trial). Experiments 1 and 2 comprised of 12 blocks, including 48 trials each, preceded by 2 practice blocks. Each block was followed by a break phase which was not limited in time but was rather controlled by participants. In half of the blocks reference trials were indicated by a red frame and comparison trials by a blue frame, and vice versa in the remaining blocks (with a counterbalanced order). Experiment 3 comprised of 8 blocks, including 40 trials each. Participants completed one practice blocks before they began the experiment. The colors used to indicate the trial types were counterbalanced *between* participants.

Baseline sEBR was also measured at the beginning of the experiment before the reference-back was introduced. In Experiment 1, sEBR were measured for 4 minutes while

participants viewed a silent short video of a waterfall. In Experiments 2 and 3, sEBR were measured for 5 minutes while participants viewed a fixation display. The only instructions given for this recording was to view the display silently. The by-condition correlations between sEBR and the other dependent variables are presented in the Supplementary.

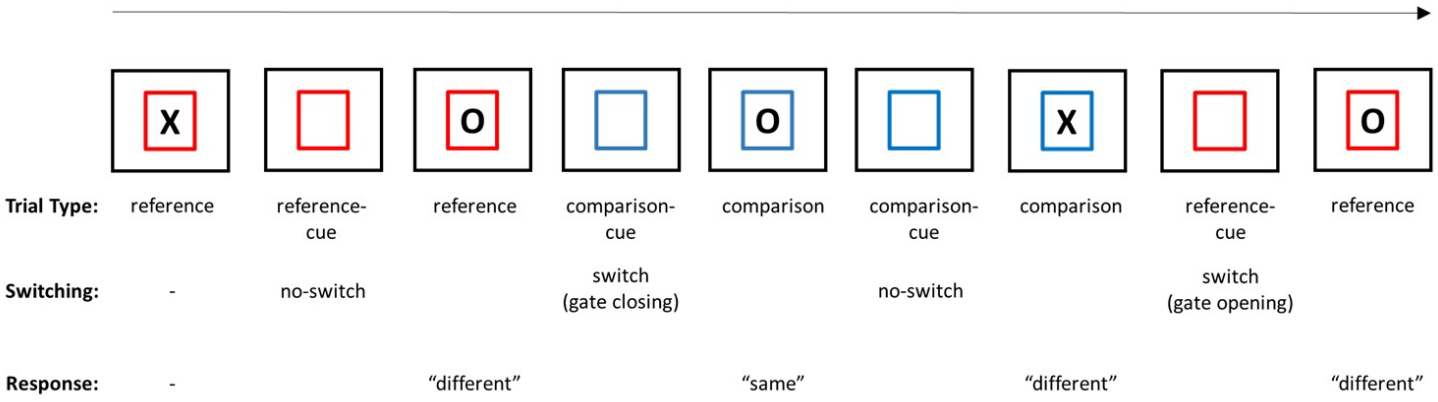

Figure 5 | The cued reference-back task. The sequence length of each trial-type was random. The fixation display was presented after response, to complete to 4.5sec. Thus, the inter-trial interval (ITI) varied as a function of the response time. The cue was presented before the stimulus as an empty colored frame for 4sec. The state of the gate and the correct response for each trial are indicted below each stimulus display.

Eye-blink recording and analysis

Eye blinks were recorded using a BioSemi Active Two system. Two external electrodes were placed above and below the right eye. Because sEBR increases in the evening[57], participants were tested between 10am and 5pm. In addition, participants were asked to avoid alcohol, nicotine, and caffeine consumption prior to the experiment, and to sleep well the night before the recording. During recordings, participants did not wear contact lenses. Importantly, they were not instructed in any manner about blinking. After recording

participants were asked what they thought we measured, and none of them suspected that eye-blinks were recorded.

The data were acquired using a 0.01–100 Hz bandpass filter and offline filtered at 1 Hz high-pass and 40 Hz low-pass (IIR Butterworth filters, attenuation slope of 12 dB/octave). The sampling rate was 256 Hz. The signal was digitized using a 24-bit A/D converter. The EOG was segmented between stimulus onset and 2sec post-stimulus in Experiment 1 and 4sec post-stimulus in Experiments 2 and 3, using EEGLAB[58]. In Experiment 3 the EOG was also segmented between cue onset and 4sec post cue. Eye blink detection was done using a MATLAB code based on the VEOG channel, created being the difference between the electrode above and under the eye, followed by manual inspection. Then, ebEBR per second was calculated for each condition.

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Figure legends

Figure 1 | The reference-back task. Trials with a red frame are reference trials and trials with a blue frame are comparison trials (see text for details). The sequence length for each trial-type was constant of 4 trials. There was a fixation display after response for 2s thus creating a 2s inter trial interval (ITI). The state of the gate and the correct response for each trial are indicted below each stimulus display.

Figure 2 | The ebEBR results of Experiment 1. Error bars represent 95% confidence intervals[59].

Figure 3 | The ebEBR results of Experiment 2. The 4sec segment length was divided to two parts, 2sec each. ebEBR as a function of Segment part, Trial-Type and Switching is presented. The effect of switching is observed only in the second half of the segment. Error bars represent 95% confidence intervals.

Figure 4 | The ebEBR results of Experiment 3. ebEBR as a function of Segment part, Trial-Type and Switching is presented. The 4sec segment length after cue presentation (a) and after

stimulus presentation (b) were divided to two parts, each of 2sec. In the cue-locked presentation (a), the switch cost is observed only in comparison trials and only in the first half of the segment. In the stimulus-locked presentation(b), the switch cost in reference trials is observed in both segment parts. Error bars represent 95% confidence intervals.

Figure 5 | The cued reference-back task. The sequence length of each trial-type was random. The fixation display was presented after response, to complete to 4.5sec. Thus, the inter-trial interval (ITI) varied as a function of the response time. The cue was presented before the stimulus as an empty colored frame for 4sec. The state of the gate and the correct response for each trial are indicted below each stimulus display.

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Authors contribution

All authors participated in the design of the study, the interpretation and writing of the final manuscript. R.R-L conducted the experiments and analyzed the data.

Competing financial interests

The authors declare no competing financial interests.