Ocular Measures During Associative Learning Predict Recall Accuracy

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Abstract

The ability to form associations between stimuli and commit those associations to memory is a cornerstone of human cognition. Dopamine and noradrenaline are neuromodulators that are critically implicated in a range of cognitive functions, including learning and memory. Eye blink rate (EBR) and pupil diameter have been shown to index dopaminergic and noradrenergic activity. Here, we examined how these ocular measures relate to accuracy during a paired-associate learning task where participants learned consistent object-location associations over eight trials. We recorded EBR, pupil diameter, and visual scanning behavior in a largely young adult sample (N=73). We examined these ocular metrics in terms of between and within participant mean centered effects using linear mixed effects modeling. In the encoding and delay epochs, we found that increased accuracy was related to increased EBR relative to the individual's own average rate within the respective epoch across the eight trials. Additionally, increased between participant mean centered EBR showed decreased accuracy in pre-trial fixation, encoding, and retrieval epochs, indicating that blinking more relative to others during these epochs was related to poorer recall. These effects of EBR on accuracy were observed even after controlling for the extent of visual scanning, which, during the delay epoch, also conferred a beneficial effect on recall accuracy. Finally, we found no effect of pupil diameter on accuracy. These results provide novel evidence supporting the utility of ocular metrics in illuminating cognitive and neurobiological mechanisms of paired associate learning.

Introduction

Associative learning underlies a wide range of cognitive abilities, and typically involves linking two previously unrelated stimuli by encoding them, binding them in memory, and subsequently retrieving them. This binding is a fundamental cognitive process. Although the frontal-hippocampal circuits that support paired object-location learning have been described (Büchel, Coull, & Friston, 1999; Diwadkar et al., 2008; Law et al., 2005; Milner, Johnsrude, & Crane, 1997), the role of neuromodulatory systems has been less thoroughly explored. Neuromodulatory systems change the excitability and potentiation of cells within the hippocampus, effectively controlling how information within the frontal-hippocampal network is processed. Dopamine (DA) and noradrenaline (NA) are two such neuromodulators that affect excitatory signaling within the frontal-hippocampal network (Hansen, 2017; Jay, 2003; Kahnt & Tobler, 2016; Palacios-Filardo & Mellor, 2019). Understanding how these neuromodulatory systems relate to associative learning may be particularly important for uncovering the physiological mechanisms of inefficient associative learning in psychiatric disorders, most notably bipolar disorder and schizophrenia (Brambilla et al., 2011; Diwadkar et al., 2008). However, *in vivo* measurement of DA and NA is a methodological challenge. In this regard, two ocular measures – eye blink rate (EBR) and pupil diameter – assume importance because they are tractable behavioral measures that provide insight into neuromodulatory activity. These ocular measures index activity of DA and NA respectively (Evinger et al., 1993; Jepma & Nieuwenhuis, 2010; Jongkees & Colzato, 2016; Koss, 1986; Wang & Munoz, 2015), and are modulated by cognitive processes that engage these neuromodulatory systems (Jongkees & Colzato, 2016; Peinkhofer, Knudsen, Moretti, & Kondziella, 2019). Here we examine how changes in pupil diameter and EBR relate to the accurate retrieval of object-location pairs across repeated presentations during an associative learning task.

Learning object-location associations relies upon functional interconnections within a network crucially involving the medial temporal lobe and frontal cortex (Büchel et al., 1999; Diwadkar et al., 2008; Law et al., 2005; Milner et al., 1997). The importance of this network for associative learning has been highlighted in humans using fMRI (Banyai, Diwadkar, & Erdi, 2011; Bunge, Burrows, & Wagner, 2004; de Rover et al., 2011) and in animal studies (Gilbert & Kesner, 2002; Langston, Stevenson, Wilson, Saunders, & Wood, 2010; McTighe, Mar, Romberg, Bussey, & Saksida, 2009; Talpos, Winters, Dias, Saksida, & Bussey, 2009). Further, a proton functional magnetic resonance spectroscopy study demonstrated that efficient learners show increased glutamate modulation in the hippocampus *early* during learning that decreases

over the course of the task as object-location associations are committed to memory (Stanley et al., 2017). Such functional excitatory dynamics are influenced by neuromodulators including NA and DA (Harley, 1987; Puig, Antzoulatos, & Miller, 2014; Tully, Li, Tsvetkov, & Bolshakov, 2007; Young, Ahier, Upton, Joseph, & Gray, 1998). Ventral tegmental area (VTA) dopamine modulates excitability within the hippocampus (Bethus, Tse, & Morris, 2010; Lisman & Grace, 2005) and affects synaptic plasticity (reviewed in Jay, 2003). Similarly, the locus coeruleus (the primary source of NA) densely innervates hippocampal structures, facilitating learning and memory through the modulation of synaptic plasticity (Harley, 1987, 1991; Kempadoo, Mosharov, Choi, Sulzer, & Kandel, 2016; McNamara & Dupret, 2017; Takeuchi et al., 2016). Ocular measures that index the activity of these neuromodulators may therefore provide insight into their role in associative learning.

Pupil dilation is tightly coupled with increased activity of the NA neurons in the locus coeruleus. This coupling occurs via direct (Koss, 1986) or indirect effects of NA activity on the Edinger-Westphal nucleus, which controls muscles in the iris, or via shared pre-synaptic input (Nieuwenhuis, De Geus, & Aston-Jones, 2011). The link between NA and pupil diameter has been validated (as reviewed in Peinkhofer et al., 2019) in pharmacology (Berridge, Gadi, Roach, & Tulloch, 1983; Ostrin & Glasser, 2004), functional imaging (Murphy, O'Connell, O'Sullivan, Robertson, & Balsters, 2014; Sterpenich et al., 2006) and single-unit recording (Gilzenrat, Nieuwenhuis, Jepma, & Cohen, 2010; Joshi, Li, Kalwani, & Gold, 2016; Rajkowski, Kubiak, & Aston-Jones, 1993) studies.

EBR, on the other hand, provides a measure of dopaminergic activity, particularly in the striatum (Groman et al., 2014; Taylor et al., 1999). Dopamine influences EBR via modulation of the endogenous spontaneous blink generator through inputs from the basal ganglia (Evinger et al., 1993). This is thought to occur via basal ganglia connections to the superior colliculus and nucleus raphe magnus which in turn modulate inputs to the trigeminal complex (Basso, Powers, & Evinger, 1996; Evinger et al., 1993; Kaminer, Powers, Horn, Hui, & Evinger, 2011; Schicatano, Peshori, Gopalaswamy, Sahay, & Evinger, 2000), providing a mechanism for striatal dopamine to affect blink rate. Coupling between resting EBR and DA signaling is further supported by findings (reviewed in Jongkees & Colzato, 2016), including human neuroimaging (Groman et al., 2014), pharmacology (Blin, Masson, Azulay, Fondarai, & Serratrice, 1990; Lawrence & Redmond, 1991), and clinical studies (Cegalis & Sweeney, 1979; Deuschl & Goddemeier, 1998; Karson, 1988; Karson, Burns, LeWitt, Foster, & Newman, 1984).

EBR and pupil dilation have been widely linked to cognitive processes, in part, because of their relationship to DA and NA activity. Increased NA activity, which is accompanied by pupil dilation, is associated with increased arousal and alertness. Baseline pupil sizes (Knapen et al., 2016) and phasic pupillary dilations (Hopstaken, van der Linden, Bakker, & Kompier, 2015) decrease with time on task, where reduced pupil responses mirror reduced arousal and poorer task performance. However, there is further nuance to the relationship between cognitive performance and both NA activity and pupillary dynamics. Two modes of LC activity have been described: phasic and tonic, (Aston-Jones & Cohen, 2005; Usher, Cohen, Servan-Schreiber, Rajkowski, & Aston-Jones, 1999), which predict on and off task attention (Unsworth & Robison, 2016). Phasic firing occurs in response to external stimuli, is associated with accurate task performance, low baseline NA activity (Aston-Jones, Rajkowski, Kubiak, & Alexinsky, 1994), small baseline (i.e. pre-trial) pupil diameter, and large task-evoked dilations (Gilzenrat et al., 2010). Tonic mode, on the other hand, is characterized by higher baseline NA firing rate, reduced phasic response to task-relevant stimuli, poor task performance (Aston-Jones et al., 1994), large baseline pupil diameter, and smaller task-evoked dilations (Gilzenrat et al., 2010).

Similarly, between-person differences in resting EBR predict performance on cognitive tasks that rely on DA transmission, such as attention (Maffei & Angrilli, 2018), inhibitory control (Den Daas, Häfner, & de Wit, 2013; Zhang et al., 2015), serial reaction time task learning (San Anton, Cleeremans, Destrebecqz, Peigneux, & Schmitz, 2018), and cognitive flexibility (Dreisbach et al., 2005; Müller et al., 2007; Sharp & Pickering, 2011; Zhang et al., 2015). Although less-studied, within-person differences in EBR across trials or in response to stimuli have also demonstrated associations with cognitive performance (Bacher, Retz, Lindon, & Bell, 2017; Drew, 1951; Oh, Jeong, & Jeong, 2012; Poulton & Gregory, 1952; Rac-Lubashevsky, Slagter, & Kessler, 2017; Siegle, Ichikawa, & Steinhauer, 2008). These effects have been characterized as tonic (differences between participants) and phasic (responses to tasks or stimuli) in the literature (Bacher & Allen, 2009; Jongkees & Colzato, 2016). As with pupil size, EBR also serves as a measure of fatigue: drowsiness is associated with faster EBR (Barbato et al., 2007; Stern, Boyer, & Schroeder, 1994).

Pupil diameter and EBR have been related to prior associative learning tasks. Phasic pupil responses are linked with lexical paired associates learning performance (Colman & Paivio, 1970), and recognition memory more broadly (Goldinger & Papesch, 2012; Heaver & Hutton, 2011; Papesch, Goldinger, & Hout, 2012; Võ et al., 2008). Rapid binding of visual stimuli and actions is associated with EBR (Aarts et al., 2012; Colzato, van Wouwe, & Hommel, 2007).

Moreover, both ocular measures have been related to associative learning in infants (Tummeltshammer, Feldman, & Amso, 2019). However, previous work has not examined the dynamics of pupil diameter or EBR over the course of associative learning (as in Stanley et al., 2017).

Here, we examine how pupil diameter and eye blink rate relate to associative learning performance using a task in which participants learned consistent object-location pairings across repeated trials. We recorded pupil diameter and EBR during four epochs within each trial – pre-trial fixation, encoding, delay, and retrieval – and related them to recall performance. We examined how performance was predicted by both between-person as well as within-person differences in these ocular measures.

Given the established links between pupil diameter and NA, EBR and DA, and that these neuromodulators critically influence functional dynamics within brain networks known to be involved in associative learning, we expected both pupil diameter and EBR to affect recall accuracy. We predicted that a decrease in NA, reflected by smaller pupil size across subjects, would impair associative learning, due to reductions in arousal and reduced promotion of memory processes. Although there is also literature that greater tonic LC activity may result in distractibility that impairs cognitive performance, we predicted that in the context of these particular task demands, fatigue effects and promoting effects of NA on regions involved in paired-associate learning would be larger. With regard to EBR, we again considered the factors that jointly and independently contribute to blink rate and that may potentially have opposing effects. Thus, we expected that across task epochs, faster EBR relative to others would predict poorer recall accuracy, given its association with fatigue. However, given DA's facilitation of learning, we also hypothesized that on learning trials with faster EBR, relative to their own average, participants would show higher accuracy.

Methods

Participants

Undergraduate students at Michigan State University (n=82) were recruited through the Department of Psychology Human Participation in Research pool provided informed consent to participate. The MSU IRB approved all study procedures. Participants were excluded for corrected visual acuity below 20/20, color blindness, not speaking English, self-reported mental illness, and use of psychotropic medications. Eight participants were determined to be noncompliant with the associative learning task and were excluded (see Data Analysis section

below for specific criterion). One participant provided incomplete data due to eye tracker dysfunction. Thus, analyses were performed on a final sample of 73 participants (52 women; mean age of 21.4 yrs., range: 18-57; $SD = 5.72$; though the 57-year-old was an outlier in terms of age, corresponding ocular and behavioral data were all within normal limits).

Apparatus and stimuli

Participants were seated in a dimly lit room with their head stabilized on a chin rest 59 cm away from a computer screen. The paradigm was presented on a 39.3 x 29.2 cm monitor (spatial resolution 1024 by 960 pixels, vertical refresh rate 85 Hz), against a black background. During the task, eye position and pupil diameter were recorded using an EyeLink 1000 Plus (SR Research, Mississauga, ON, Canada). Stimulus presentation was controlled with MATLAB (The MathWorks, Natick, MA) using the Psychophysics (Brainard, 1997) and EyeLink (Cornelissen, Peters, & Palmer, 2002) toolboxes.

Associative learning task

The task was adapted from previous studies (Burn & Hutcheon, 1949; Diwadkar et al., 2016; Diwadkar et al., 2008), and is depicted in **Figure 1**. Participants learned nine object-location associations over the course of eight trials. The object-location pairings were consistent across learning trials. Each trial consisted of four epochs: fixation, encoding, delay, and retrieval. Each trial began with a drift correction procedure where participants fixated an annulus, and pressed the space bar. Successful drift correction initiated the onset of the fixation epoch. During the fixation epoch (9s), participants fixated on a white cross presented at the center of the screen. During the encoding epoch (27s), participants were sequentially presented with nine equifamiliar and mono-syllabic objects (Snodgrass & Vanderwart, 1980), each at a reserved location in a 3 x 3 grid (3s per object). A given object's location on the grid did not change across trials, but the order in which objects were presented within each trial was pseudorandomized and consistent across participants. During the delay epoch (27s), participants were presented with an empty 3 x 3 grid to which they were asked to remain oriented. During the retrieval epoch (27s), participants were presented with the 3 x 3 grid, and sequentially cued to each location with a red square for 3s. During this cue, participants were asked to verbalize the object that was presented at that location. Participants responded with "no" if they were unable to recall, or guess the cued object. Verbal responses were recorded by the experimenter. Prior to the advent of the first trial, participants were presented with a practice trial, in which the true task stimuli were replaced by color coded emoticons.

Figure 1. Associative Learning and Memory Paradigm. (a) This schematic illustration portrays the trial structure, which consists of four discrete epochs, each 27s in duration: fixation, encoding, delay, and retrieval. During the encoding epoch, nine stimuli (e.g. pen, shoe) are sequentially presented for 3s in each location on the grid. During the retrieval epoch, participants were cued by a filled-square to verbalize their response at each location. There were eight total learning trials (b) Nine equi-familiar stimuli were shown during the encoding epoch of each of the 8 learning trials. The white number in the corner of the cell represents the order at which these stimuli were presented within a given learning trial. Object-location pairings remained consistent across trials, however, the sequence was pseudorandomized by trial and left consistent across participants.

Data analysis

Performance on the associative learning task, pupil diameter, and blinks were analyzed using MATLAB (The MathWorks, Natick, MA). Statistical analyses were performed using R and the lme4 toolbox (Bates, Sarkar, Bates, & Matrix, 2007; R development core team, 2013).

Associative learning

Accuracy was computed for each of the eight trials. Based on prior validation in associative learning studies using similar paradigms (Banyai et al., 2011; Buchel, Coull, & Friston, 1999) learning curves were iteratively fit to the accuracy data for each individual using a negatively accelerated response function of the form:

$$[\text{accuracy} = a - be^{-c(\text{time})}],$$

Where a is the asymptote, b is the difference between the initial performance and the asymptote, c is the slope of the curve (larger c values indicate faster learning), and e is Euler's number. Data from eight participants resulted in poor fits (adjusted R^2 for the best fitting model was negative, suggesting non-incremental performance) and these were excluded from further analysis (resulting in a final sample of 73 participants).

Ocular measurements

Pupil diameter was measured during the fixation and delay epochs, while EBR was measured during all four epochs. Pupil diameter is confounded by eye position when using video-based eye trackers. Thus, samples where eye position fell beyond a two-degree visual angle square centered on central fixation as well as samples coinciding with blink and saccade events were removed from analysis. Pupil diameter from only the final two seconds from the fixation and delay epochs were averaged, in line with previous studies (Gilzenrat et al., 2010). There were no subjects for whom mean EBR or mean pupil diameter across the eight trials exceeded three standard deviations from the group mean in any epoch. There were isolated instances within trials during which eye-tracker dysfunction produced missing samples for an entire epoch. Across participants and learning trials, pupil data was not viable in the final two seconds of twelve fixation epochs and one delay epoch, and eye blink data was not recorded or useable in two fixations epoch, one encoding epoch, one delay epoch, and four retrieval epochs.

Blinks were marked by the Eyelink online event parser using their proprietary algorithm based on several consecutive missing pupil samples. This algorithm has been independently shown to accurately identify blinks (Ehinger, Groß, Ibs, & König, 2019). To further ensure data quality in our blink measurements, we removed blinks outside of the normal range of blink duration (less than 80 ms or more than 900 ms in line with reported blink durations in Ehinger et al., 2019). After removing outliers, the mean blink latency was 220.21 ms ($SD = 51.53$ ms). EBR (blinks/min) was calculated for each epoch (fixation, encoding, delay, and retrieval) in each participant by dividing the total number of blinks in that epoch by the epoch duration and multiplying by 60 seconds.

Blink rate and pupil size are sensitive to eye movements and, thus, visual scanning (Evinger et al., 1994; Fogarty & Stern, 1989; Manning, Riggs, & Komenda, 1983; Mathôt, van der Linden, Grainger, & Vitu, 2015; Watanabe, Fujita, & Gyoba, 1980). Furthermore, visual scanning has been found to affect visual learning processes (Brandt & Stark, 1997; Brockmole & Irwin, 2005; Laeng & Teodorescu, 2002; Spivey & Geng, 2001). Thus, to ensure that putative effects of

blinks or pupil size on accuracy were not due to differences in scanning behavior, we also computed a measure of visual scanning (see Fogarty & Stern, 1989), incorporating these values into our statistical models. Scanning behavior was operationalized as the summed distance in visual angle between fixations, and was computed for each epoch within each learning trial.

Statistical analyses

Learning over time was modeled at the group level using a linear mixed effects model predicting accuracy as the combination of linear and quadratic effects of time:

$$[\text{accuracy} \sim t + t^2 + (1|ID)]$$

Where t is the mean centered trial, t^2 is the squared, mean centered trial, and $1|ID$ reflects participant level random effects.

To assess how ocular measures within each epoch related to learning across trials, linear mixed effects models predicting accuracy from both trial and ocular measures were run. Data across learning trials from each epoch were analyzed separately with distinct models for pupil data and EBR data. Therefore, two models were run for pupil data (fixation and delay epochs), and four for EBR (all task epochs). Each of these models took a similar form:

$$\text{Accuracy} \sim t + t^2 + \text{wpO}_e + \text{gmO}_e + \text{wpD}_e + \text{gmD}_e + \text{wpO}_e:t + \text{gmO}_e:t + \text{wpD}_e:t + \text{gmD}_e:t + (1|ID)$$

Where t is the mean centered trial, t^2 is the squared, mean centered trial, wpO_e is the within-person mean centered ocular measure (blink rate or pupil diameter) from the epoch of interest, gmO_e is the grand-mean centered ocular measure from the epoch of interest, wpD_e and gmD_e are the within-person mean and grand-mean centered distances between fixations for the given epoch, included as a control for differences in scanning behavior. Both trial and the squared value of trial were included to account for the typical positive linear trend in accuracy with learning, and the eventual flattening out of that curve as maximal learning is achieved. Grand-mean centered ocular measures were included to test whether differences *between* individuals during a particular epoch in the ocular measure of interest predict accuracy (e.g. do people who blink more, on average, during the delay period show greater accuracy?). Within-person centered ocular measures were included to test whether differences *within* an individual during a particular epoch predict accuracy (e.g. when people blink more during the delay period, relative to their own mean blink rate during the delay period, do they show greater accuracy?).

The interaction terms between the grand-mean centered effects and time were included to determine whether changes in ocular metrics over time were related to corresponding changes in accuracy. The random intercepts by participant were included to account for between participant random variance.

Results

In the following section, we first describe behavioral performance during the associative learning task. We then present models predicting accuracy on the associative learning task based on pupil diameter and EBR.

Associative Learning

Accuracy over time, averaged across participants and fitted with a negatively accelerated response function is depicted in **Figure 2**. We found significant linear and quadratic effects of learning trial on performance (see **Table 1**): participants became more accurate with repeated presentation of the object-location pairs and this increase in accuracy was asymptotic across trials.

Table 1. *Percentage Correct Predicted by Experimental Trial.*

Random Effects	Variance	Std. Dev.	N.	Observations
Participant (Intercept)	195.6	13.99	73	584
Fixed Effects	Estimate	Std. Error	T-value	P-value
Intercept	35.26	2.81	12.56	<.001
Trial	17.04	1.16	14.66	<.001
Trial ²	-1.23	0.13	-9.74	<.001

Pupil Diameter

We examined whether pupil diameter in the fixation or delay epochs was related to learning while controlling for distance between fixations. In both epochs, trial showed significant ($p < 0.001$) linear and quadratic influences on accuracy. Neither within-person, nor grand-mean centered pupil diameters, in either the fixation (**Table 2A**) or delay epoch (**Table 2B**) predicted accuracy (all $ps > 0.162$). The grand-mean centered pupil diameter also did not interact with time in its influence on accuracy in either epoch (all $ps > 0.638$). Scanning behavior (defined by distance between fixations) did not relate to accuracy in the fixation epoch; however, in the delay epoch the grand-mean centered distance between fixations was significantly related to accuracy ($p = 0.024$) and showed a significant interaction with time ($p = 0.022$). This suggests that more scanning behavior during the delay epoch was related to increased accuracy, but that this relationship decreased with repeated presentation of object-location pairs (see **Figure 3a**).

Figure 2. Associative learning performance. The graph shows the mean proportion of correct responses per trial fitted to a negatively accelerated learning function of the form: $\text{performance} = a \cdot b e^{-c(\text{time})}$.

Table 2. Percentage Correct Predicted by Change in Pupil Diameter.

A. Fixation Epoch				
Random Effects	Variance	Std. Dev.	N.	Observations
Participant (Intercept)	205.8	14.34	73	572
Fixed Effects	Estimate	Std. Error	T-value	P-value
Intercept	80.57	1.77	45.34	<.001
Trial	17.09	1.17	14.68	<.001
Trial ²	-1.23	0.13	-9.69	<.001
Within Person Centered Pupil Diameter	0.00	0.00	1.08	0.281
Within Person Centered Distance Between Fixations	0.05	0.06	0.89	0.377
Grand mean centered pupil diameter	0.00	0.00	0.26	0.792
Grand Mean Centered Distance Between Fixations	-0.04	0.06	-0.72	0.474
Grand Mean Centered Pupil Diameter*Trial	0.00	0.00	0.38	0.705
Grand Mean Centered Distance Between Fixations* Trial	0.00	0.00	0.58	0.565
B. Delay Epoch				
Random Effects	Variance	Std. Dev.	N.	Observations
Participant (Intercept)	195.6	13.99	73	583
Fixed Effects	Estimate	Std. Error	T-value	P-value
Intercept	80.70	1.74	46.50	<.001
Trial	16.51	1.17	14.06	<.001
Trial ²	-1.16	0.13	-9.09	<.001
Within Person Centered Pupil Diameter	0.00	0.00	1.40	0.162
Within Person Centered Distance Between Fixations	-0.01	0.02	-0.29	0.776

Grand Mean Centered Pupil Diameter	-0.00	0.00	-0.33	0.745
Grand Mean Centered Distance Between Fixations	0.05	0.01	2.28	0.024
Grand Mean Centered Pupil Diameter*Trial	0.00	0.05	0.47	0.638
Grand Mean Centered Distance Between Fixations* Trial	-0.01	0.05	-2.29	0.022

Figure 3. Interactions of trial with distance between fixations in the delay epoch and with eye blink rate in the fixation epoch. A) The interaction between trial and summed distance in visual angle between fixations in the delay epoch shows a stronger positive relationship with accuracy on earlier trials (darker red) that is reduced and disappears by the final trial (light yellow). **B)** The interaction between trial and eye blink rate in the fixation epoch shows a negative relationship with accuracy that is stronger on earlier trials (darker red) but disappears by the final trial (light yellow).

Eye Blink Rate

Descriptive statistics for EBR in each of the four task epochs are presented in **Table 3**. We examined the relationship between EBR and accuracy in all four epochs, controlling for scanning behavior (see **Table 4**). In all four epochs we identified linear and quadratic effects of trial (all $ps < 0.001$).

In the fixation epoch, we found a significant effect of grand-mean centered EBR ($p = 0.035$) and a significant interaction between grand-mean centered EBR and trial ($p = 0.029$). Participants who blinked more during fixation, on average, had overall poorer performance (lower accuracy). The interaction with time indicated that this relationship between EBR and accuracy was most prominent in the early learning trials (**Figure 3b**). No other effects were significant.

In the encoding epoch, we found a significant effect of grand-mean centered EBR ($p = 0.027$) such that more blinks than average were associated with lower accuracy. We also observed an effect of within-person centered EBR ($p = 0.012$), but in the opposite direction. On trials where participants blinked more relative to their average blink rate across the encoding period, they showed *increased* accuracy. Critically, this was the case when including scanning behavior and time in the model. No other effects were significant.

In the delay epoch, we found a significant effect of within-person centered EBR on accuracy ($p = 0.005$). On trials where participants blinked more relative to their average blink rate across the delay period, they showed increased accuracy. Interestingly, we also found a significant effect of grand mean centered distance between fixations ($p = 0.014$). Those individuals who had a greater average distance between fixations on average had increased accuracy. This is noteworthy, because there were no stimuli on the screen during the delay epoch.

In the retrieval epoch, we again found that grand-mean centered EBR was significantly related to accuracy ($p = 0.036$) such that blinking more, relative to the group average, led to decreased accuracy. No other effects were significant.

The persistent finding across three epochs that increased grand-mean centered EBR is related to decreased accuracy suggests that blinking more than average is related to poorer

performance. Conversely, the findings in the encoding and delay epochs demonstrated that on trials where participants blinked more relative their own individual mean, accuracy was better. These effects of within-person centered blinks remained even after conservative Bonferroni correction for the number of statistical tests performed when examining the effect of EBR on accuracy (corrected $\alpha = 0.0125$). Additionally, we demonstrated that during the delay epoch there was a relationship between scanning behavior and accuracy, suggesting that more visual scanning during this epoch when no stimuli were present predicted better accuracy.

Table 3. *Descriptive blink statistics in each epoch*

Epoch	Mean (blinks/min)	Standard Deviation
Fixation	25.29	15.11
Encoding	24.55	12.97
Delay	32.68	23.28
Retrieval	31.73	13.20

Table 4. *Percentage Correct Predicted by Eye Blink Rate in the Four Epochs*

A. Fixation Epoch

Random Effects	Variance	Std. Dev.	N.	Observations
Participant (Intercept)	197.0	14.04	73	582
Fixed Effects	Estimate	Std. Error	T-value	P-value
Intercept	80.64	1.74	46.31	<0.001
Trial	17.16	1.17	14.65	<0.001
Trial ²	-1.24	0.13	-9.75	<0.001
Within Person Centered Distance Between Fixations	0.01	0.06	0.011	0.916
Grand Mean Centered Distance Between Fixations	0.01	0.06	0.20	0.844
Within Person Centered EBR	0.22	0.14	1.58	0.117
Grand mean centered EBR	-0.31	0.15	-2.13	0.035
Grand Mean Centered EBR*Trial	0.03	0.02	2.19	0.029
Grand Mean Centered Distance Between Fixations* Trial	-0.00	0.01	-0.32	0.747

B. Encoding Epoch				
Random Effects	Variance	Std. Dev.	N.	Observations
Participant (Intercept)	182.5	13.51	73	583
Fixed Effects	Estimate	Std. Error	T-value	P-value
Intercept	80.64	1.68	47.88	<0.001
Trial	16.76	1.22	13.71	<0.001
Trial ²	-1.19	0.13	-9.07	<0.001
Within Person Centered Distance Between Fixations	0.01	0.03	0.43	0.667
Grand Mean Centered Distance Between Fixations	0.03	0.03	1.22	0.227
Within Person Centered EBR	0.42	0.17	2.55	0.012
Grand mean centered EBR	-0.35	0.16	-2.24	0.027
Grand Mean Centered EBR*Trial	0.00	0.02	0.14	0.890
Grand Mean Centered Distance Between Fixations* Trial	-0.00	0.00	-0.44	0.659
B. Delay Epoch				
Random Effects	Variance	Std. Dev.	N.	Observations
Participant (Intercept)	188.6	13.73	73	583
Fixed Effects	Estimate	Std. Error	T-value	P-value
Intercept	80.67	1.71	47.29	<0.001
Trial	16.02	1.18	13.63	<0.001
Trial ²	-1.12	0.13	-8.79	<0.001
Within Person Centered Distance Between Fixations	-0.02	0.02	-1.06	0.292
Grand Mean Centered Distance Between Fixations	0.06	0.02	2.50	0.014
Within Person Centered EBR	0.32	0.11	2.83	0.005

Grand mean centered EBR	-0.08	0.10	-0.86	0.391
Grand Mean Centered EBR*Trial	-0.01	0.01	-0.93	0.354
Grand Mean Centered Distance Between Fixations* Trial	-0.00	0.00	-1.69	0.093
D. Retrieval Epoch				
Random Effects	Variance	Std. Dev.	N.	Observations
Participant (Intercept)	193.0	13.89	73	580
Fixed Effects	Estimate	Std. Error	T-value	P-value
Intercept	80.60	1.73	46.68	<0.001
Trial	17.46	1.18	14.83	<0.001
Trial ²	-1.28	0.13	-9.98	<0.001
Within Person Centered Distance Between Fixations	-0.01	0.02	-0.33	0.740
Grand Mean Centered Distance Between Fixations	0.03	0.02	1.11	0.271
Within Person Centered EBR	0.28	0.16	1.80	0.074
Grand mean centered EBR	-0.33	0.16	-2.12	0.036
Grand Mean Centered EBR*Trial	0.03	0.02	1.54	0.125
Grand Mean Centered Distance Between Fixations* Trial	-0.00	0.00	-0.16	0.877

Discussion

The aim of the present experiment was to use ocular measures as a window into the functional mechanisms that subserve associative learning by recording pupil diameter and EBR during a paired object-location associative learning paradigm. We additionally examined visual scanning, as it has been related to both visual learning, as well as EBR and pupil size. Given the prior literature on associative learning we hypothesized that within-person and between-person differences in EBR would differentially affect learning given the relationships between

EBR and both dopaminergic activity (Jongkees & Colzato, 2016; Taylor et al., 1999) and fatigue (Stern et al., 1994), as well as reported dopaminergic modulation of hippocampal-mediated learning (Jay, 2003; Palacios-Filardo & Mellor, 2019). We also hypothesized that sustained pupil diameter would relate to accurate task performance by virtue of its relationship with NA-mediated arousal processes. Our findings provided mixed support for these predictions. First, we found that during both the encoding and delay periods, when participants blinked more *relative to their own average blink rate* across all eight encoding or delay epochs, they showed increased accuracy. Second, when participants blinked more, *relative to others*, during the fixation, encoding, and retrieval epochs they showed poorer task performance. These results were observed even after controlling for the extent of visual scanning, which can itself lead to changes in EBR. Third, participants who engaged in more visual scanning during the delay period showed increased accuracy. Lastly, pupil size did not predict accuracy in either the pre-trial fixation period or the delay period. Here, we discuss the potential cognitive and neurobiological implications of the aforementioned findings.

We found that increased within-person mean-centered EBR during the encoding and delay epochs predicted accurate behavioral performance. This result indicates that on trials in which participants blinked more relative to their average blink rate – averaged across the eight learning trials for the encoding and delay epochs separately – they were more accurate. Prior research on the relationship between EBR and learning has primarily focused on learning mediated by striatal D2 receptors (e.g. Slagter, Georgopoulou, & Frank, 2015). One possibility is that within-person increases in EBR on a trial-by-trial level may reflect phasic increases in dopaminergic activity that have the potential to influence hippocampal circuits thought to underlie this type of paired associates learning (de Rover et al., 2011; Diwadkar et al., 2016; Kim, Heath, Kent, Bussey, & Saksida, 2015; Sommer, Rose, Weiller, & Büchel, 2005). Such an effect would be most likely during the encoding and delay phases when binding of objects and their locations in the hippocampus would transpire.

Another possibility is that increased EBR relative to one's own average blink rate during the encoding and delay periods—when information is being registered and maintained—reflects the engagement of working memory processes. Phasic bursts of dopamine provide gating signals for afferents to prefrontal cortex, enhancing representation of new information (Miller & Cohen, 2001; Müller et al., 2007) and working memory (Floresco & Phillips, 2001). These gating mechanisms have been proposed to rely on striatal dopamine to determine what relevant information is allowed into working memory (Frank, Loughry, & O'Reilly, 2001) through the

enhancement or inhibition of information within cortical-striatal-thalamic-cortical loops. Increased striatal activity during delay periods (Hikosaka, Sakamoto, & Usui, 1989) and striatal dopamine (Landau, Lal, O'Neil, Baker, & Jagust, 2009) have both been linked to working memory performance. Indeed, in a previous study increases in event-based EBR were associated with increased working memory demands (Rac-Lubashevsky et al., 2017). Therefore, increased relative EBR – especially during the delay phase when participants are likely to be actively maintaining information – may reflect working memory processes that engender later recall accuracy. This explanation may be reconciled with the extensive literature demonstrating hippocampal involvement in associative learning given the role of visuo-spatial working memory as a buffer for accessing and manipulating hippocampal representations (Axmacher et al., 2010; Treisman & Zhang, 2006). Given that our task required repeated study and testing of the same object-location associations the interaction of frontal and medial temporal systems suggested by this result aligns with prior findings on the testing effect (Wing, Marsh, & Cabeza, 2013) and with effective connectivity analyses in a comparable associative learning paradigm (Banyai et al., 2011).

In contrast to effects seen for within-person changes in EBR, increased grand mean centered EBR during the fixation, encoding, and retrieval epochs predicted worse behavioral performance. That is, people who blinked more on average during the fixation, encoding, and retrieval epochs had *lower* recall accuracy. Although at face value, these results appear to be in conflict with findings that people who blink more relative to their own blinking rate have better recall accuracy (during encoding and delay periods), we consider here the multiple influences on and functions of blink rate. For example, EBR is also correlated with drowsiness: individuals who are more tired show increased frequency of blinks (Barbato et al., 2007; Stern et al., 1994). If this is the cause of the increased blink rate then participants with higher blink rates may have been more fatigued leading to decreased engagement with the task. In line with this idea, prior studies have found that higher EBR was associated with reduced cognitive stability or increased distractibility and therefore lower accuracy (Dreisbach et al., 2005; Tharp & Pickering, 2011). We furthermore found an interaction between grand mean-centered EBR in the fixation epoch and trial: greater EBR is negatively associated with accuracy in early learning trials, but that relationship disappeared by the end of the experiment. This would suggest that putative EBR-indexed fatigue is more detrimental to accuracy early in learning.

Inter-individual differences in EBR may also reflect tonic differences between participants in underlying dopaminergic signaling. In line with this interpretation, a prior study

that administered a dopamine agonist in order to increase tonic dopaminergic signaling showed worse associative learning within the group taking the agonist (Breitenstein et al., 2006). Our results, in line with their interpretation, therefore may suggest that overall higher tonic levels of dopamine reduce long-term potentiation in the hippocampus by changing the balance between D1/D2 receptor modulation of AMPA channel insertion into glutamatergic neurons (Breitenstein et al., 2006; Sun, Zhao, & Wolf, 2005; Wolf, Mangiavacchi, & Sun, 2003). Furthermore, prior work using a delayed spatial memory task showed increased EBR was associated with decreased working memory accuracy in a load dependent fashion using a spatial working memory task similar to that used here (Lee et al., 2018). Controlling for sleepiness or time of day effects in future studies and collecting independent working memory assessments may help tease apart these competing interpretations.

Finally, increased visual scanning in the delay epoch was associated with increased accuracy. This effect is reduced over trials with a stronger relationship in the beginning of the experiment and no effect by the final trial. This result aligns with literature suggesting that visual rehearsal during delay periods is associated with improved performance on spatial memory tasks (Brandt & Stark, 1997; Brockmole & Irwin, 2005; Laeng & Teodorescu, 2002; Spivey & Geng, 2001). For example, Brockmole and Irwin (2005) identified differences in accuracy across participants in integrating information displayed in dynamic locations on the screen. These differences were related to participant's eye movements during a delay, implying that differences in visuo-spatial rehearsal supported accurate performance. Our finding that distance between fixations during the delay period was related to accuracy – especially at the beginning of the experiment – raises the possibility that active rehearsal of the display during the delay period facilitated learning.

The current findings should be interpreted in the context of some limitations. First, we were only able to reliably quantify pupil diameter during the pre-trial fixation and delay period when the eye was stable and centrally fixated. During the other task epochs, eye movements, which confound measures of pupil diameter when using video-based eye trackers, were directed across the screen. As a result, we are not able to capture phasic pupillary responses to task stimuli or look at pupil diameter during encoding or retrieval epochs. We removed pupil measures during eyelink identified blinks and saccades; however, the persistent effects of these ocular events over time may have obscured an effect related to cognition (Knapen et al., 2016). Second, our sample was not balanced across gender (with more women participants). Despite this, we did not observe any significant gender differences in ocular measures, or any

differences between women who were and were not using birth control¹, the imbalance could still be potentially problematic. First, there are reported gender differences in EBR, with women blinking more than men, however, more have found otherwise (Jongkees & Colzato, 2016). Furthermore, we did not account for women's menstrual cycles or hormonal birth control either of which may affect EBR, given that D2 receptor availability covaries with estrogen levels (Yolton et al., 1994). Third, the link between EBR and DA activity remains an ongoing area of research. Two recent positron emission tomography (PET) studies have advised caution when inferring DA activity from resting EBR (Dang et al., 2017; Sescousse et al., 2018). Their results cast doubt on the relationship between EBR and striatal dopamine function at rest, rather than cases where dopaminergic signaling is induced by experimental demands, which consequently influences EBR. However, the link between such task related EBR changes and dopamine is a claim largely reliant on behavioral rather than neural data. Fourth, the timeframe over which our blink rates were calculated (on the order of seconds) may have compromised the reliability of these measurements (Jongkees & Colzato, 2016). Finally, there is evidence for non-linear relationships between cognitive task performance and both pupil diameter (Aston-Jones & Cohen, 2005) and EBR (Akbari Chermahini & Hommel, 2012; Cools & D'Esposito, 2011), such that intermediate values predict optimal cognitive performance under certain sets of demands. Our models unfortunately could not accommodate these quadratic effects, which may explain the lack of significant effects of pupil diameter on recall accuracy. Non-linear effects of ocular metrics on performance should be tested in future studies with larger sample sizes.

In conclusion, we found that eye blink rate and visual scanning behavior during specific task epochs predicted recall accuracy on a paired-associates learning task. Although blinking more relative to others, was related to worse accuracy, blinking more relative to one's own blink rate (during encoding and delay periods) showed better accuracy. Further we found that visual scanning during the delay was associated with accuracy early in the experiment suggesting that visuo-spatial rehearsal may benefit accuracy in this type of paired object-location associative learning. The link between eye blink rate and dopaminergic activity permits mechanistic explanations at the level of neuromodulation, and thus, our results provide novel insight into the temporal dynamics of dopamine during associative learning. These findings lay the framework for future study in neuropsychiatric populations to gain insight into the nature of associative learning dysfunction.

¹ We did not observe any differences in pupil diameter or EBR during any of the task epochs in our sample between either (1) men and women or between (2) women who did (n=12) and did not (n=40) report oral contraceptive use in our sample (all p's > 0.13).

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