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The Effects of Mild Ocular Surface Stimulation and Concentration on Spontaneous Blink Parameters

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Abstract

Purpose—This exploratory, pilot study compared the effects of concentrating on a visual task and a very mild ocular surface air stimulus on multiple blink parameters.

Methods—Ten subjects participated in this study. There were 2 visits, one with an ocular surface air stimulus (AS) and one without (NS). The AS was set at a level barely perceptible by subjects (approximately 0.6m/sec at the eye). At each visit, subjects performed a high (HC) and low concentration (LC) task. Blinking was tracked and tear-film breakup (TBU) was monitored simultaneously to measure blink parameters, including the interblink interval (IBI), blink amplitude, duration, maximum velocity and TBU before and after each blink.

Results—During the HC tasks, IBI was significant higher and blink duration was lower (repeated measures ANOVA, $p < 0.05$) than the LC tasks. The IBI in the AS-LC condition was significantly lower and less variable than in the NS-HC condition, whereas blink duration showed the opposite effect (Hotelling T^2 test, $p < 0.005$). There was high individual variation in correlations between blink amplitude and maximum velocity. The area of TBU was not significantly correlated with any blink parameter.

Conclusions—The lack of correlation between TBU and blinking suggests that many blinks are stimulated by internal controls, rather than direct stimulation of the ocular surface by TBU. This pilot study suggests that even very mild ocular surface stimulation produces opposite effects on the timing and duration of the blink, when compared to concentrating on a visual task. The HC task tends to decrease blink frequency and duration, presumably to minimize interruption by the eyelids, whereas mild ocular surface AS increased blink frequency and duration, most likely to increase protection of the ocular surface.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Keywords

Blinking; Tear film instability; tear film break-up; dry eye

INTRODUCTION

Dry eye is a common condition that affects millions in the US(1, 2) and worldwide.(3, 4) It is considered to be a multifactorial disease of the tear film and ocular surface and is associated with symptoms of ocular discomfort and visual disturbance.(5) Low blink rate has been identified as a potential risk factor for the development of dry eye because it can result in increased evaporative loss from the tear film.(5–9) This in turn may lead to increased tear film hyperosmolarity and instability,(10) which are considered core mechanisms of dry eye.(5) In addition, the blink acts to spread the tear film over the ocular surface,(11) so that understanding its interaction with the tear film and the stimuli involved in the blink response may be important in the etiology of the dry eye condition.

Controls over spontaneous blinking, which includes all but voluntary or reflex blinks,(12) remain controversial.(7, 13, 14) Blink rate is known to be highly variable and depends on many factors, including cognitive state,(15–17) ocular surface input(7, 14, 18) and central dopamine level.(19–21) Visual tasks requiring concentration, such as playing computer game or reading are known to slow the blink rate,(6, 14, 16, 22) sometimes markedly,(23) whereas stimulation or irritation of the ocular surface can increase the blink rate.(6, 7) The apparent excitatory input of the ocular surface sensory mechanisms on blinking is of special interest due to its putative effect in dry eye,(24) where sensory input from the unstable tear film(10) and corneal sensory nerve damage or functional changes may be expected to affect blinking.(25–28)

Many of the studies investigating the effect of the ocular surface on human blink rates have involved relatively dramatic or sizeable changes in ocular surface input, such as abrogating ocular surface sensation with anesthetic, which decreases the blink rate.(7, 13, 29) Likewise, damage to corneal nerves following refractive surgery is associated with a lower blink rate,(30) whereas placing a contact lens on the eye, which could be considered an irritant to the ocular surface, has been reported to increase the blink rate.(31) The dry eye condition is associated with an increased blink rate,(6, 32) perhaps due to stimulation by the unstable tear film,(6) although few studies have investigated the connection between the tear film and blinking or devised experimental conditions with a relatively mild stimulus to the ocular surface.

Recently, animal studies have suggested that blinking is controlled by a spontaneous blink generator based in the trigeminal complex, which is modulated, but not controlled, by corneal afferents.(27) While it is clear that substantial blockage, stimulation or alteration of corneal afferents by instilling an anesthetic,(7, 13, 29) wearing a contact lens(31) or neural damage(30) are sufficient to effect changes in blinking, little is known about the effect of lesser ocular surface stimuli on blinking in humans. For this reason, we designed a pilot study to explore the effects of a relatively mild air stimulus to the ocular surface while varying the level of attention to a visual task with simultaneous monitoring of tear film

instability to examine any associated changes in blinking. In addition, we investigated multiple blink parameters, rather than focusing on blink rate only, because factors such as blink amplitude and duration may also be affected by attention or concentration on a visual task(31, 33) and ocular surface stimulation.(34)

METHODS

Subjects

The study was conducted at the Borish Center for Ophthalmic Research at the Indiana University School of Optometry, Bloomington, Indiana and adhered to the tenets of the Declaration of Helsinki. It was approved by the Institutional Review Board at Indiana University and informed consent was obtained from each subject prior to beginning the study.

Ten subjects, some with and some without dry eye symptoms, as measured by the Dry Eye Questionnaire (DEQ),(35) were recruited for this study. We recruited subjects with a range of dry eye symptoms because this pilot study was exploratory in nature, with an eventual, future goal of studying the dry eye condition. However, subjects who showed significant corneal staining (>Grade 2, Oxford Scale⁽³⁶⁾) were excluded because corneal staining could potentially be a confounding factor affecting the ocular surface sensory response to tear film instability.(26, 37, 38) Subjects with ophthalmic conditions other than dry eye or systemic disease were also excluded.

Before beginning the study, subjects were informed that the experiment was designed to observe the tear film while they were doing tasks. Monitoring of blinking was not disclosed until subjects completed the study to prevent self-conscious or unnatural blinking.

Experimental Procedures

This study consisted of two visits: with (AS) and without (NS) an air stimulus. In order to also vary the level of attention to a task, each subject performed a high (HC) and low concentration (LC) task at each visit. The order of the visit and task (AS-HC, AS-LC, NS-HC and NS-LC) was randomly determined. The LC task consisted of listening to classical music while looking straight ahead. The HC task involved playing a computer video game (Tetris®), which the subject viewed through a beam splitter. Each task lasted 2.5 minutes and there was a 15-minute break between the tasks.

The AS was generated using a small electronic fan. The air stimulus level was set based on the results of a small pilot study with 25 subjects in which the distance of the fan to the eye was altered until it was perceived as a barely detectable light breeze that was not considered bothersome. An average final distance of 50cm from the eye was chosen, which yielded a measured air velocity of 1.34 mph (0.6m/s) at the eye. According to the Beaufort scale for wind speed (<http://www.spc.noaa.gov/faq/tornado/beaufort.html>), which includes categories 0 (calm) to 12 (hurricane), the velocity we used is at the low end of the “light air” category 1 (0.3–1.5m/s) and is insufficient to move wind vanes or tree leaves. It is considered to be very mild, even less than a “light breeze” (category 2).

After filling out the DEQ,(35) subjects were seated behind a Zeiss biomicroscope system (8x magnification) with two custom-fitted cameras, which were used to simultaneously record upper lid movement (Point Grey Research, 250Hz) and tear film stability (Mistubishi HS-U69, 30Hz). In order to track eyelid positions during blinking, a 2mm diameter reflective white dot (3M®) was positioned on the margin of the right upper lid. Two microliters of 2% fluorescein dye was instilled into the inferior bulbar conjunctiva using a micropipette for an initial grading of corneal staining (Oxford Scale⁽³⁶⁾) and tear film stability assessment during the experiment. At the air stimulus visit, the recording was started one minute after the onset of air stimulus to allow subjects become familiar with the stimulus. Only the right eye was tested and the left was held shut by the subject.

At the end of the study, the Schirmer's I tear test (without anesthetic) and fluorescein tear break up time (TBUT) were performed to assess the level of dry eye for study subjects.

Blink Analysis

From each trial, a one-minute recording was extracted for analysis that began at 30 seconds into the 2.5 minute task. During each blink, the vertical movement of the reflective tape on the upper eyelid was tracked through the blink process and parameters including blink amplitude, maximum velocities and durations of the down and up phases, and total duration were calculated using a custom MATLAB® (The Mathworks™, Natwick, MA) program. For each subject, the amplitude of a complete or 100% blink was calibrated prior to each task by asking the subject to blink fully and measuring the maximum vertical closure of the eyelid during that blink. The amplitude of all subsequent blinks for that subject was expressed as a percent ratio of the original, calibrated, full blink. The maximum velocity was calculated as the fastest eyelid movement during the down and up phases and the absolute maximum velocity was calculated in millimeters per second. When comparisons were made with blink amplitude, which was a relative measure calibrated by each individual's full blink, the maximum velocity was also standardized by a calibrated full blink and expressed as a percent ratio of the full blink per second. The blink duration was defined as beginning when the lid velocity first reached 10% of its maximum velocity during the down phase and ended when it reached 10% of its maximum velocity during the up phase.(39) The interblink interval (IBI) was calculated as the time from the fullest extent of one blink to the fullest extent of the next blink.

Tear Break-up Analysis

Images of the tear film (single frame) immediately before and after each blink were obtained from the digital recordings to calculate the area of tear break-up (TBU) as a percent of the exposed corneal area before and after each blink using methods described previously.(6, 40, 41) Briefly, color fluorescent images were converted to grayscale, and the region of total exposed cornea (region of interest) outlined. A custom MATLAB® program was used to calculate the percentage of TBU using a threshold pixel intensity set by the investigator for each trial. The investigator was unaware of the subject or trial information.

Statistical Analysis

Repeated-measures ANOVA with Bonferroni corrected post hoc testing were used to test attention and air stimulus main effects and their interaction for all blink parameters. Hotelling T^2 tests were used to make multivariate comparisons of mean and standard deviation metrics of the IBI, blink amplitude and down phase duration during study conditions. A paired t test was used to compare the area of TBU before and after blinks. The Pearson's correlation coefficient was used to determine correlations between blink parameters and with the area of TBU.

RESULTS

Subjects

The average age ($\pm$ standard deviation) of study subjects was 34 ± 14 years (range: 22–57 years). Six were female and 4 were male. The median DEQ-5 score(42) was 9 (range: 0–16), and half of the subjects reported a previous dry eye diagnosis on the DEQ.(35) Initial corneal and conjunctival staining was negligible in all subjects, with a median of 0 and a range of 0–1 on the Oxford Scale.(36) The average ($\pm$ standard deviation) Schirmer's I tear test and TBUT were 17.5 ± 5.9 mm/5min (range: 8–24mm) and 11 ± 10 sec (range: 2–58 sec), respectively. Given the combination of symptoms and clinical signs, previously diagnosed dry eye subjects would be categorized as mild to moderate (Grade 2), according to the DEWS modified Delphi guidelines.(43)

Individual Examples

Figure 1 displays all blinks during the 60 sec trial for Subject 1, who did not report a previous dry eye diagnosis (DEQ-5 score=0, Schirmers= 20mm/5min, TBUT=58sec). Under baseline conditions (Figure 1A), blinks were relatively similar and regularly spaced, although none were full amplitude. In comparison, increased concentration (Figure 1B) resulted in fewer blinks of lesser amplitude, with an increased IBI variability, while the air stimulus (Figure 1C) increased the amplitude and regularity of blinking. The combination stimulus (Figure 1D) produced a greater range of blink amplitude and duration and an increased IBI variability.

Subject 2, who had been previously diagnosed with dry eye (DEQ-5 score=16, Schirmers= 8mm/5min, TBUT= 3sec), displayed fuller blinks than Subject 1 under most conditions (Figure 2). As with Subject 1, blinking slowed with concentration (Figure 2B), increased with the air stimulus (Figure 2C), and the IBI became more irregular, with more incomplete blinks of shorter duration with the combination stimulus (Figure 2D). Some blinks, often categorized as cluster blinks(44) or blink oscillations(25, 45) occurred very close together (Figure 2: closed arrows). Some blink traces were greater than 100% amplitude (Figure 2: open arrows), as defined by our measurement calibration system. These blinks appeared to be due to the subject squeezing the eye tightly shut, perhaps in response to the irritation of the air stimulus, which exceeded the original calibration blink.

Blink Parameters

Interblink Interval (IBI)—Table 1 lists aggregate data for each blink parameter and Figure 3 shows error bar plots of selected parameters for each individual subject. The IBI under baseline conditions (NS-LC) can be converted to an average blink rate of 12 blinks/min, which is well within the range of previous studies.^(6, 16) Regardless of the AS, the average IBI under HC was significantly increased compared to LC ($p = 0.017$). In comparison, AS appeared to decrease the IBI regardless of concentration levels, although the difference did not reach statistical significance ($p = 0.067$). There was no interaction between concentration and air stimulus main effects ($p > 0.05$). As Figure 3A shows, IBI varied within and among subjects under most of the experimental conditions except for the AS-LC condition under which variability was relatively low.

As Figure 3 shows, both the mean and within subject variability of the IBI appeared to be affected by the conditions tested in this study. Figure 4A plots the relationship between the mean and standard deviation of the IBI, comparing the effect of concentration (NS-HC) to the air stimulus condition (AS-LC). There was little overlap between the points, with the AS-LC points clustered at the bottom left of the graph due to their short IBIs and low variability, whereas the NS-HC points showed largely higher IBI's, greater variability and were more scattered. Each square dot is the centroid for the AS-LC (closed) and NS-HC (open) conditions, and the ellipses summarize the pooled within-group scatter for each test condition. The Hotelling T^2 shows that the centroid of the mean and standard deviation under NS-HC and AS-LC conditions were significantly different ($p < 0.001$). This graph illustrates that air stimulation and concentrating on a visual task produce almost opposing effects on the IBI and its variability.

Blink Amplitude—The average blink amplitude was similar among conditions (Table 1), but there were some individual differences among tasks (Figure 3B). Some subjects showed decreased amplitude and increased variability when concentrating on the computer task (NS_HC), but there was no significant concentration, air stimulus main effects or interactions ($p > 0.05$).

Figure 4B compares the mean and standard deviation of the NS-HC and AS_LC and shows that there was extensive overlap between the points, suggesting neither concentration nor the air stimulus markedly effected blink amplitude. The Hotelling T^2 confirms this, demonstrating that the centroid of the mean and standard deviation under the NS-HC and AS-LC conditions were not significantly different from each other ($p = 0.843$).

In agreement with previous results,⁽⁶⁾ the majority of blinks in this study were incomplete. Blinks with amplitude below 100% ("partial blinks"), comprised 79% of the total number of blinks. However, 71% of partial blinks fully covered the pupil. Blinks with amplitudes above 100%, appeared to be due to occasional forceful squeezing of the eyes shut during the blink so that the tracking dot excursion exceeded that of the initial calibration for a full blink, thus registering as $>100\%$ (see the arrows in Figure 2C). Most (74%) of these blinks were in the subjects with a previous dry eye diagnosis, suggesting that more frequent, forceful blinks with squeezing of the eyelids may occur in dry eye. However, because this

investigation involved only a few dry eye subjects, this observation should be tested in future studies.

Blink Duration—As others have found,(12, 39) the average duration of the down phase of the blink (Table 1) was significantly shorter compared to the up phase (paired t-test, $p<0.001$). Regardless of air stimulus, down phase, up phase and total blink duration were significantly shorter during HC tasks compared to LC tasks ($p= 0.010$, 0.044 and 0.013 , respectively). No significant air stimulus main effect was found. There were no interactions between task concentration and air stimulus effects.

As with IBI, the variability varied among conditions, in addition to the mean (Figure 4C). In this case, the down phase blink duration with air stimulus (AS-LC) was greater and more variable than with concentration on a task (NS-HC). The Hotelling T^2 shows that the centroid of the mean and standard deviation under the NS-HC and AS-LC were significantly different ($p= 0.0016$), with few points in each group overlapping. These data suggest that concentrating on a visual task is associated with short duration blinks, perhaps to minimize disrupting concentration and or vision by the lids, whereas air stimulation tends to be associated with blinks of longer duration and higher variability, perhaps to protect the ocular surface.

Maximum Velocity—As Table 1 shows, the down phase maximum velocity was much higher than that in the up phase, which agrees with previous reports.(12, 46, 47) When conditions were compared, there were no concentration or air stimulus main effect on down or up phase velocity, but there was a significant interaction between concentration and air stimulus for down phase maximum velocity ($p=0.021$). During the high concentration tasks, the down phase maximum velocity increased with the air stimulus, while the opposite occurred with the low concentration tasks, suggesting that the change in maximum velocity depends on both attention and the air stimulus.

Correlation between Blink Amplitude and Maximum Velocity

As in previous studies, there was significant correlation between blink amplitude and maximum velocity when all the data was pooled together ($r= 0.64$ $p<0.001$), suggesting that fuller blinks tend to have a higher maximum velocity.(12, 46) However, individual subjects varied in the slope and degree of correlation between these parameters. To illustrate this point, Figure 5 shows the relationship between blink amplitude and maximum velocity for all subjects in this study under all conditions. We divided subjects by previous dry eye diagnosis because we noted that some dry eye subjects showed a much greater variability in this relationship, compared to subjects who had not previously been diagnosed with dry eye. Pooling all the conditions together, the mean slopes $\pm$ standard deviation for non-dry eye and previously diagnosed dry eye subjects were 0.15 ± 0.06 and 0.22 ± 0.15 , respectively. Although not conclusive in this pilot study, these data suggest that individual correlations should be examined, rather than the common practice of pooling data from multiple subjects, because dry eye or other subjects may show differing relationships among common blink parameters.

Tear Film Instability and Its Correlation with Blinking

Table 2 shows the average TBU area before and after blink under the four study conditions. The amount of TBU was significantly decreased after blinking compared to before for all conditions ($p=0.001$). However, it should be noted that significant TBU often remained after the blink, especially inferiorly. This appeared related to the high number of incomplete blinks (see the section on Blink Amplitude) that failed to fully wet the cornea.

The high variation of TBU before the blink during NS-LC was due to one subject who only blinked five times and who had high TBU area before each blink. With the data from this outlier removed, the TBU average and standard deviation for NS-LC was $5.12 \pm 4.49\%$. There was no statistically significant difference in the percent TBU among the four conditions studied.

Correlations between blink parameters and TBU before or after the blink were low (r ranges from -0.004 to 0.254). (6, 48) Figure 6 shows the relationship between one blink parameter, the IBI, and TBU before the blink. Similar results were found when data were separated by condition or individual. Although some blinks followed large amounts of TBU over the cornea, many blinks occurred without TBU. In addition, many blinks were incomplete and thus failed to fully clear TBU.

DISCUSSION

This was a pilot study designed to explore the effects of concentration and a relatively mild surface stimulus on multiple blink parameters, which are not often measured on human subjects. (7, 13, 29–31) We found that an ocular surface air stimulus that was considered barely noticeable produced an increased frequency and regularity of blinking, whereas concentrating on a visual task produced less frequent and more irregularly spaced blinks. Blink duration was decreased when concentrating, presumably to minimize distraction by the eyelid during the task. These data support the hypothesis that blink parameters, including frequency and duration, are modulated by even mild surface input and cognitive levels. (6, 7, 22, 31, 45)

Blink frequency, as measured by the IBI in this study, increased even with the very mild air stimulus used in this study. Similar results have been found with other presumed ocular surface stimuli, such as wearing contact lenses (31) or having dry eye. (6, 7) The presumed explanation is that ocular surface input, which is in the form of an inadequate tear film in dry eye, tear film evaporation from air stimulus, or a contact lens, acts to stimulate the ocular surface, resulting in increased blinking. While it is difficult to compare the stimuli used in this and previous studies to each other, these results suggest that a wide range of ocular surface inputs, from the barely noticeable air stimulus used in this study to severe dry eye affect blinking, although the degree may vary widely. (6, 7, 13, 31)

The variability of the IBI was also affected by the air stimulus used in this study. As the rate of blinking increased with the very mild air stimulus, so did the regularity of blinking. (32) Similar results were found in animal models, where stimulation of the ocular surface or supraorbital nerve were associated with an increased spontaneous blink rate and enhanced

regularity of the blink pattern.(27) Irritation of the ocular surface is known to produce trigeminal reflex blink excitability and extra blinks, termed blink oscillations, that occur at a relatively constant interval after an initial reflex blink.(45) Blink oscillations occur in dry eye animal models, leading to the hypothesis that increased blink rate and regularity in dry eye may be an adaptive modulation of the blink response to provide a better tear film.(27, 45)

While the air stimulus used in this study increased the rate and regularity of blinking, concentration on a visual task produced the opposite effect (Figures 4A and C). Many have shown a decreased blink rate with concentration and increased attentional state,(16, 23, 48, 49) but the temporal distribution also becomes more irregular and tends to cluster(31) during short-term cognitive encoding of information. In a study of spontaneous blink rates in patients with vegetative conditions, the irregularity of blinking increased with clinical improvement, suggesting that irregular blinking is associated with recovery of consciousness and cognitive thought processing.(50) In this study, subjects playing a computer game and showed a wide variability in both blink rate and regularity (Figures 1, 2 and 3), which may, in part, reflect the degree of attention each subject paid to the game. We did not measure the degree of attention to the visual task in this study, which may have introduced some differences in blink response among subjects. However, the decreased rate and regularity of blinking found among many subjects presumably occurred due to increased cognitive activity with some longer periods between blinks, theoretically to increase information processing.(22)

According to previous studies, concentration reduces blink amplitude as well as decreasing the blink rate,(31, 33) presumably to minimize distraction by the eyelids. In this study, there was no statistically significant reduction in blink amplitude while playing the game, although individual subjects did show a reduction in blink amplitude (Figure 1B). This discrepancy might be due to small sample size of current study and the different experimental design. Regardless of differences in blink amplitude among subjects and conditions, many blinks (79%) were partial in this study. However, even though incomplete blinks were common, 71% of blinks covered more than two thirds of the corneal surface, so that most covered the pupil, providing good tear film over the pupil area. These results agree with a previous study from our laboratory(6, 31) raising questions as to why partial blinking is so common. It is possible that the primary purpose of the blink is to spread the tear film over the pupil to ensure a good optical surface for the eye. This could explain why partial blinks and the resulting inferior tear break-up are so commonly found in this and our previous study.(6) In addition, we have also previously shown that the tear film was more stable among dry eye subjects following a partial compared to a full blink.(51) Thus, although incomplete blinks are often considered undesirable, their relative frequency raises questions about their contributions to vision, attentional state and tear film stability.

Blink duration also appeared to be affected by attentional state and ocular surface input in this study. The high concentration task produced significantly shorter duration blinks with less variability than the air surface stimulus (Figure 4C), presumably for the purpose of minimizing interruption by the eyelid during the task. Even in the combined condition of air stimulation and concentration, blink duration was still short and regular (Figure 3C). Blink

duration was longer and much more variable with the air stimulus, suggesting that some longer blinks occurred to protect the ocular surface from irritation. Although few have studied spontaneous blink duration under different conditions, increased blink duration is associated with trigeminal excitability, which may occur in response to supraorbital nerve stimulation(12, 34) or decreased dopamine levels.(20, 25)

Others have shown a tight correlation between amplitude and velocity, suggesting that, as the blink becomes fuller, it is faster(12). We found a similar result when all data was pooled, as in many previous studies. However, this association was more variable when individual subjects were examined, especially in some subjects who had been previously diagnosed with dry eye (Figure 5). As the slope of this relation is considered to be an indicator of motor neuron activity of eyelid muscles,(52) this suggests blinking kinetics in individual subjects may differ, perhaps relating to altered ocular surface inputs in dry eye. Some studies have shown that dry eye subjects have desensitized ocular surfaces(53), although others have shown hypersensitivity in dry eye subjects(38, 54). While this study was small, exploratory and involved only a few subjects, it may be useful to further examine individual correlations between blink parameters in future studies, rather than pooling data.

Some have suggested that the ocular surface controls blinking because stimulating the surface or a poor tear film in dry eye increases the blink rate, while anesthetic decreases the blink rate(7). In this and a previous study, we examined the relationship between tear film break-up and blinking, reasoning that an unstable tear film with break-up should stimulate increased blinking. In both studies, tear break-up occurred while subjects played the video game, especially inferiorly, and often was not fully cleared by the frequent partial blinks. However, we were unable to find a direct relationship between tear break-up and blinking in either study.(6) One possibility may be that we measured only tear break-up and not significant thinning. Both are likely to occur in an unstable tear film,(55) producing transient increased tear hyperosmolarity, which would be likely to stimulate surface neurons.(10) Another possibility is that mild corneal irritation stimulates the trigeminal to increase blinking,(27, 45) thus masking the initial stimulus. In addition, we directed the air flow to stimulate the ocular surface, but stimulation may also have occurred to the eyelids during blinking. The air flow may also have increased tear film evaporation which could increase cooling or tear film osmolarity, both of which would stimulate ocular surface neurons.(26, 56) Thus, the nature of the air stimulus and the ocular surface controls over blinking remain unclear.

This pilot study evaluated multiple blink parameters to compare the effects of concentration on a visual task and an air stimulus that was designed to be barely noticeable by subjects. It was an exploratory study designed to raise questions to be addressed in future studies. Even with a small sample size and using subjects with a range of dry eye symptoms, we found that even a mild air stimulus produced opposing effects on blink frequency and duration when compared to concentration on a task. Although we did not design the study to compare dry eye to normal subjects, the subjects in this study with a previous dry eye diagnosis tended to show more variable blinking kinematics compared to non-dry eye. These results suggest that individual subjects may respond differently, so that future studies are needed to

address the ocular surface versus cognitive controls over blinking and their importance in the dry eye condition.

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Figure 1.

Blink traces (left), blink timing, amplitude (vertical bars), the area of TBV before blink (dots) and the average IBI $\pm$ standard deviation during 60 sec trial (right) for Subject 1 under four experimental conditions (A–D). Blink amplitude is displayed on a reverse axis to mimic the direction of the lid during the blink.

Figure 2.

Blink traces (left), blink timing, amplitude (vertical bars), the area of TBU before blink (dots) and the average IBI $\pm$ standard deviation during 60 sec trial (right) for Subject 2 under four experimental conditions (A–D). Blink amplitude is displayed on a reverse axis to mimic the direction of the lid during the blink. Open arrow points to the blink amplitude over 100%; filled arrows point to cluster blinks that occurs with short intervals

Figure 3. Error plots of the IBI (A), blink amplitude (B), and down phase duration (C) for all subjects under four test conditions.

Figure 4.

Scatter plots of mean versus the standard deviation of the NS-HC (open circles) and AS-HC (filled circles) of the IBI (A), blink amplitude (B), and down phase duration (C) for all the subjects. The square dots are the centroids for each condition and the ellipses summarize the pooled within-group scatter for each test condition.

Figure 5. Blink amplitude versus maximum down phase velocity under four conditions for subjects with and without a previous diagnosis of dry eye.

Figure 6.
Scatter plots of IBI and area of TBU before the blink for all subjects under all conditions.

Table 1

Averages $\pm$ standard deviations for all blink parameters under different conditions.

Concentration	Air stimulus	IBI (sec)	Blink amplitude (%)	Blink Duration (msec)		Maximum velocity (mm/sec)	
				Down phase	Up phase	Down phase	Up phase
Low	No (NS-LC)	5 $\pm$ 3	86 $\pm$ 17	93 $\pm$ 23	155 $\pm$ 23	168 $\pm$ 61	92 $\pm$ 26
	Yes (AS-LC)	3 $\pm$ 1	80 $\pm$ 18	100 $\pm$ 24	169 $\pm$ 54	155 $\pm$ 75	88 $\pm$ 33
High	No (NS-HC)	6 $\pm$ 3	75 $\pm$ 23	82 $\pm$ 13	142 $\pm$ 30	140 $\pm$ 57	83 $\pm$ 33
	Yes (AS-HC)	5 $\pm$ 3	80 $\pm$ 20	81 $\pm$ 17	138 $\pm$ 26	161 $\pm$ 58	91 $\pm$ 23

Gray shading: Significant main effect, HC versus LC, $p < 0.05$, repeated measures ANOVA

Table 2

The percent area of TBU before and after the blink (mean± standard deviation) under different conditions.

Condition	Before blink (%)	After blink (%)
NS-LC	8.26±10.63**	2.31±2.19
NS-HC	5.89±4.72**	2.85±2.52
AS-LC	5.56±4.76**	3.25±2.64
AS-HC	4.20±3.36**	2.50±2.13

**
significant difference between before and after the blink (paired t test, p=0.001)