Published in final edited form as:

Behav Brain Res. 2010 September 1; 212(1): 1–11. doi:10.1016/j.bbr.2010.03.011.

Neural Correlates of Fast Pupil Dilation in Nonhuman Primates: Relation to Behavioral Performance and Cognitive Workload

R.E. Hampson, Ioan Opris, and S.A. Deadwyler

Department of Physiology & Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27157

Abstract

Pupil dilation in humans has been previously shown to correlate with cognitive workload, whereby increased frequency of dilation is associated with increased degree of difficulty of a task. It has been suggested that frontal oculomotor brain areas control cognitively-related pupil dilations, but this has not been confirmed due to lack of animal models of cognitive workload and task-related pupil dilation. This is the first report of a wavelet analysis applied to continuous measures of pupil size used to detect the onset of abrupt pupil dilations and the frequency of those dilations in nonhuman primates (NHPs) performing a trial-unique delayed match to sample (DMS) task. An additional unique finding shows that electrophysiological recordings in the same animals revealed correlated firing of neurons in frontal cortex with different components of pupil dilation during task performance. It is further demonstrated that the frequency of fast pupil dilations (but not rate of eye movements) correlated with cognitive workload during task performance. Such correlations suggest that frontal neuron encoding of pupil dilation provides critical feedback to other brain areas involved in the processing of complex visual information.

Keywords

oculometrics; pupil diameter; frontal cortical neurons; cognitive demand; delayed-match-to-sample task; behavior; electrophysiology; perievent histogram; monkey; nonhuman primate; rhesus

Introduction

Oculometrics provide several interesting measures of subject attention, information processing and cognition. Measures of eye and eyelid movement (e.g. eyeblink speed and frequency, saccade speed, duration and frequency, fixation time and pupil diameter) can be correlated with performance in tasks with varying cognitive demands [20,24,25,32,48] in accordance with tracking of eye position to determine how objects in the visual field are examined and detected [18]. Measures of pupillary dilation have recently been used to assess cognitive load by recording rapid changes in pupil diameter [3,45,46,48,49]. Such changes correlate significantly with alterations in mental effort of subjects required to attend to multiple interfering stimuli

© 2010 Elsevier B.V. All rights reserved.

Corresponding Author: Sam A. Deadwyler, Ph.D. Dept. of Physiology & Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27157-1083, Phone: 336-716-8540, Fax: 336-716-8628, sdeadwyl@wfubmc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

[28,41], suggesting that pupil dilations indicate cognitive processing of visual features, and not merely reactions to the luminosity of the stimulus or environment.

To date, correlation of cognitive load with pupillometry has been examined mostly in humans and not in animal models. Oculometric measures (including pupillometry) are often only performed on humans engaged in complex tasks and are combined with empirical or subjective measures of cognitive workload [19,30,45], and in some cases correlated with brain imaged measures [8,9,17].

Recent studies have applied advanced statistical analyses [21,47-50] to oculometric and pupillometric measures to derive sensitive indicators of cognitive workload. Rutishauser and Koch [38] proposed a visual search model for comparing neural activity of V4 and frontal neurons with feature extraction of visual stimuli, a model that requires recording of single neuron processing in primate brain. Here we report the first instance of pupillometric recording in nonhuman primates (NHPs) in a behavioral task previously demonstrated to be sensitive to changes in cognitive workload [10,16,35]. Recordings from single neurons in the frontal cortex (FCx) of NHPs are shown to be differentially synchronized to fast pupil dilation events, thereby providing important insight into the neural basis of the established correlation between pupillometry and cognitive workload in humans.

Methods

Animals

Four adult NHPs (male rhesus monkeys - *Macaca mulatta*), weighing (8.0-11.0 kg) were employed as subjects, they were housed in stainless steel cages in temperature and humidity controlled colony rooms with lighting maintained on a 0600:1800 day:night cycle and fed a daily diet of monkey chow supplemented by fresh fruit. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Wake Forest University, and performed in accordance with established practices as described in the National Institutes of Health and Public Health Service policy on humane care and use of laboratory animals.

Behavioral Training and Testing

Each monkey was trained to perform a visual delayed-match-to-sample (DMS) task for juice reinforcement and tested daily 5 times per week. Animals were seated in a primate chair 1.5 meters in front of an LCD-front-projection screen and performed 150–300 trials per 60-90 min DMS test session [10,16,35]. They were trained to move a cursor into 25 cm clipart images on a 1.0 meter $\times$ 1.0 meter video projection screen by positioning the hand on a counter mounted to the chair, within a two dimensional coordinate system that was video tracked by a fluorescent marker attached to the back of hand. The DMS task consisted of a Sample and a Match phase in which an image presented in Sample phase was responded to and then a delay period of 1-90 s duration (selected at random) interspersed with the screen blanked and only the cursor illuminated. At termination of the delay interval, 1-5 non-match or “distracter” images were displayed together with the original (Sample) image constituting the Match phase of the task in which placement of the cursor into the correct “Match” phase image (i.e. same image as presented in the Sample phase) produced an immediate juice reward via a sipper tube positioned next to the animals’ mouth. All displayed clip art images were unique to a particular trial, and no image was used on more than one trial within an entire session. Animals received a portion of their normal daily fluid intake (60-100 ml/kg) during the behavioral testing session followed by the remainder upon return to the home cage. All animals were trained to a stable baseline performance level of 70-80% correct over all trials, however, as shown previously performance accuracy varied directly with difficulty or “cognitive workload” determined directly by the

number of images presented in the Match phase [10]. Sets of clip art images were routinely changed frequently to maintain the trial-unique feature of each session.

Pupillometry

Due to the varying visual stimuli of the task, a pupillometry analysis that was not affected by background luminosity was required for this analysis. The analysis used here was first reported and described by Marshall et al. [27,29,46,50], applied to human studies. The technique identifies pupil dilations with rapid rise times (approx 100 ms) associated with recognizing or categorizing visual stimuli, and has previously been shown to correlate to conditions of increased cognitive effort in humans [26,28,29].

Eye movements and pupil diameter were continuously monitored from NHPs performing the DMS task (Figure 1) using video recording (EyeLink 1000, SR Research, Ontario, Canada) from an IR reflective glass (Figure 1A) placed at a 45 ° angle between the monkey and the computer display. Each animal's head was fixed in position during DMS task performance to allow consistent recording of the right eye. Software provided with the 'EyeLink' Oculometry system digitized images of the pupil at 250-1000 samples/sec (Figure 1A), computed pupil diameter and direction of gaze, and categorized saccades, blink and gaze fixation events (Figure 1B). An analysis "waveform" was constructed from continuous raw pupil diameter recordings, with saccades and blinks removed (shaded regions, Figure 1B), leaving only the recordings during fixation of eye position. The resulting pupil diameter record was then decomposed using a Daubechies DB8 wavelet (Figure 1C). The "Level 1 Details" of decomposition represent fast (< 100 ms) changes in pupil diameter corresponding to the *onset of pupil dilation*. The Level 1 Details were thresholded to retain only pupil dilation events resulting in greater than 10% change in pupil diameter (Figure 1D). The frequency of pupil dilation events was computed as number of fast dilations/sec and compared to other DMS task events. Individual timestamps of fast dilation events derived from the wavelet analysis were recorded and used for correlation with task-related events. Since dilation artifacts could be induced by blinks and saccades, the pupil diameter was filtered *prior to wavelet analysis* to exclude the influence of saccades or blinks.

Surgery

Four male rhesus monkeys were surgically prepared via craniotomy and for access cylinders (25 mm anterior to interaural line, 10 mm lateral). Craniotomy sites for neural recording were selected to overlie the stereotaxic coordinates (25 ± 3 mm anterior to interaural line, 11 ± 3 mm lateral midline) of the superior arcuate sulcus of the frontal cortex (FCx, areas 6 – and 8 – the frontal eye fields) [6] as certified from a monkey brain atlas [34] and normalized by individual MRIs for each monkey. All surgical procedures related to electrode positioning, recording and maintenance were performed under sterile conditions. Animals were sedated with ketamine (10 mg/kg), intubated, and maintained throughout surgery with isoflurane (1-3%, to effect) and oxygen. An access cylinder (Crist Instruments) was placed on the skull above the craniotomy site and fixed to the skull with screws and dental cement to allow daily placement of microelectrodes into the FCx. Each animal received antibiotics (Baytril, 25 mg/kg) for 7 days after surgery, and buprenorphine (0.7 mg/kg) as needed for pain. Animals were allowed to recover from the procedure for 10–15 days before resuming testing.

Electrode Positioning and Recording

At the beginning of daily experiments, the recording cylinder was opened, cleaned and disinfected. Recording electrodes (2-4 etched 200 μ m tungsten wires, 5-10 μ m tip diameter) were positioned in the center of the craniotomy cylinder such that electrode tips would enter the brain within ± 3 mm of stereotaxic coordinates for Area 8 of FCx (25 mm anterior, 11 mm lateral, [34]). The electrodes were introduced into brain and slowly advanced (0.25 mm/minute)

via an Alpha-Omega Electrode Positioning System or Narishige micromanipulator to the appropriate depth for FCx recording (1-5 mm ventral to surface of brain) while neural activity was monitored to determine final positioning. Identification of recorded brain regions was based on MRI-confirmed stereotaxic placement of the craniotomy cylinders, and electrode tracks within those cylinders, which were compared to appropriate coordinates in an NHP stereotaxic atlas [34]. It was not possible to mark every cell recording locus with a microlesion because of possible damage from the number of cells recorded from the same region in successive sessions as well as expected utilization of the same NHPs in other laboratory studies. However, histological analyses in some animals confirmed recording tracks in the superior arcuate sulcus consistent with Area 8. Once the electrode was positioned in a cortical cell layer as determined by increased spontaneous firing, single neurons were isolated electronically and recorded during a 60-90 minute behavioral testing session. Only FCx neurons that did not exhibit responses to saccades were tested in the DMS task. Upon completion of the recording session, electrodes were removed, and the recording cylinder disinfected and sealed.

Single neuron waveforms were discriminated and analyzed with a Multineuron Acquisition Processor (Plexon, Inc.) and NEX software (NeuroExplorer, Inc.). Perievent histograms (PEHs) and rastergrams of cortical neuron activity were constructed for Sample and Match phases of the task in addition to histograms synchronized to wavelet-identified fast dilation events via timestamps of each recorded neural waveform. Standard scores ($z = [\text{peak firing rate} - \text{baseline firing rate}] / [\text{standard deviation of baseline firing rate}]$) were calculated for each event. Cell types were identified by the presence (or absence) of significant ($z > 3.09$, $p < 0.001$) increases or decreases in peak or sustained firing the PEHs [16] related to both DMS and pupil dilation events.

Effects of Sleep Deprivation

Previous studies from this laboratory demonstrated that sleep deprivation increases cognitive workload as measured by choice accuracy and response latency measures of DMS performance [10]. Sleep deprivation consisted of 30-36 hours of continuous wakefulness, verified using EEG recordings of sleep architecture, supervised continuously by laboratory personnel. Animals were maintained awake using methods described previously [10,35]. After testing animals were returned to their home cage to resume the normal daily routine. There were no residual effects of the 30-36 hr sleep deprivation regimen on DMS performance the day afterward sleep deprivation testing at which time animals had returned to their normal sleep cycles. A minimum interval of 10-14 days, mandated by IACUC regulations, was interspersed between sleep deprivation episodes for each animal. To provide an intermediate manipulation for increasing cognitive load in the task, DMS testing was also conducted on different occasions in the late afternoon when animals were “sleepy” at a time immediately prior to onset of the usual night cycle with lights off at 1600 hrs.

Results

Neural Correlates of Pupil Dilation

Four NHPs, previously trained in the above-described multi-object, visual delayed match to sample (DMS) task [10,16], were tested following positioning of recording electrodes in the FCx (see Surgery). Figure 1 illustrates the pupillometry recording and wavelet analysis used to identify fast pupil dilation events during behavioral performance as described above in Methods. Figure 2 shows firing of a single FCx neuron synchronized to each pupil dilation event (top traces) that occurred during 100 trials within a single DMS session (as shown in Figure 2B). The traces at the top of Figure 2A illustrate the change in pupil diameter (pixels) synchronized to *onset* of the pupil dilation (vertical dashed line) identified by the wavelet analysis (Figure 1C). Wavelet synchronized mean firing rate increased from a baseline of 1.6

± 0.3 Hz at 250 ms prior to dilation onset, to a mean rate of 3.6 ± 0.5 Hz at 250 ms following dilation, with a peak firing rate of 3.9 ± 0.7 Hz. Both peak and mean firing rates were significantly different from baseline (Peak: $Z=7.7$, $p<0.001$; difference between post dilation and baseline rate: $F_{(1,284)} = 16.21$, $p<0.001$). Figure 2B shows that both frequency of dilations and neural firing peaked soon after image presentation in both Sample and Match phases, and steadily declined until the respective behavioral response. Frequency of Dilation and neural firing rate were significantly correlated across this interval ($r=64$, $F_{(19,284)} = 3.26$, $p<0.001$).

Dilation related activity was recorded from FCx neurons in each of the four NHPs during performance of the DMS task over 10-15 sessions ($n=101$ cells). Figure 3 shows responses of FCx neurons classified by significant changes in firing frequency during the occurrence of pupil dilations identified by the wavelet analysis (Figure 1D), and sorted into categories on the basis of increased or decreased firing in relation to onset of fast pupil dilation. The plot in the upper half of Figure 3A depicts individual mean firing patterns as overlapping line tracings (Individual Neurons) for a set of FCx neurons ($n=28$) that exhibited significant increases ($F_{(19,173)} = 4.06$, $p<0.01$) in mean ($\pm$ SEM) firing rate 250 ms *prior to* the onset of pupil dilation (lower histogram). Figure 3B shows the firing patterns and mean firing rates of FCx neurons ($n=24$) that exhibited significant brief increases in firing ($F_{(19,151)} = 3.55$, $p<0.001$) 100ms after dilation onset (Dilation *ONSET*) with a smaller secondary peak at 200ms ($F_{(19,151)} = 2.12$, $p<0.01$) after dilation onset (asterisks in Fig 3B). Figure 3C shows significantly elevated mean firing rate after onset of pupil dilation ($F_{(19,113)} = 5.39$, $p<0.001$) in a different set of FCx neurons ($n=16$) that maintained significantly elevated firing levels for 500-700 ms before returning to baseline firing rate (sustained, *Sust.*). A final group of FCx neurons ($n=17$) shown in Figure 3D (*Inhibited*) exhibited significant reductions or inhibition of firing rate relative to baseline ($F_{(19,113)} = 3.18$, $p<0.001$) at the onset of pupil dilation, for approximately 100 ms then reversed and increased rate toward baseline firing levels.

FCx Neurons in Different Categories Fire during Separate Phases of Pupil Diameter Change

The mean firing patterns of FCx neurons in the 4 categories shown in Figure 3A-D (colored traces) are plotted in Figure 3E along with the corresponding mean change in pupil diameter (black) before and after (± 0.4 s) their respective dilation episodes. The mean firing rate traces for the 4 different categories of neurons are shown in Figure 3E (*Pre*, *Onset*, *Sust.*, *Inhib.*) paired with the mean pupil diameter changes associated with the firing of that type of neuron. The respective neural discharge patterns correspond to different phases (i.e. types of) pupil dilation in that *Pre* and *Sust.* neurons were associated with similar types of dilations from a steady baseline, while *Onset* neurons were associated with more rapid but briefer dilations and *Inhib.* neuron inhibition was associated with dilations that immediately followed a brief (0.5 s) constriction.

Correlation of Frontal Cortical Activity during DMS Task Performance with Pupil Dilation

As expected from prior investigations [10,16,35] the firing of neurons in FCx increased to presentation of the Sample and Match images during DMS performance. The rasters and histograms in Figure 4 illustrate the frequency of pupil dilation events (upper) and firing (lower) during the Sample (left) and Match phases (center and right) synchronized to image presentation (solid vertical line at 0.0 s) for an *Onset* FCx neuron. In the Sample phase (1-image) the frequency of fast pupil dilation events increased significantly from 2.2 ± 0.4 Hz (0.5 sec pre-Sample baseline) prior to presentation to 5.3 ± 0.7 Hz (0-0.5 sec) following Sample presentation ($F_{(1,97)} = 8.91$, $p<0.01$), then declined to 4.1 ± 0.4 Hz within 500 ms. Firing of the this *Onset* neuron significantly increased from a pre-Sample baseline of 1.2 ± 0.3 Hz to a peak firing of 6.2 ± 0.6 Hz ($F_{(1,123)} = 12.4$, $p<0.001$) 0.5 sec after Sample presentation and declined back to baseline firing rate within 400 ms (Figure 4A). On trials with 3 images in the Match phase the mean frequency of pupil dilations significantly increased from pre-

presentation baseline (1.8 ± 0.8 Hz) to a peak level (7.9 ± 0.7 Hz) immediately after image presentation ($F_{(1,97)} = 11.17$, $p < 0.001$), then declined significantly (4.4 ± 0.6 Hz, $F_{(1,97)} = 9.48$, $p < 0.001$) over the next 600ms to baseline levels (Figure 4B). The firing rate of the same *Onset* neuron increased significantly higher (relative to the same baseline) to a peak of 9.4 ± 0.7 Hz ($F_{(1,123)} = 15.7$, $p < 0.001$) following presentation of 6 images in the Match phase, and declined rapidly to baseline firing level (1.3 ± 0.5 Hz). In Match phases with 6 images, pupil dilation frequency increased from pre-presentation baseline (2.1 ± 0.7 Hz) to a peak level (9.1 ± 0.8 Hz) immediately after image presentation ($F_{(1,97)} = 13.42$, $p < 0.001$), then declined slightly (5.8 ± 0.8 Hz, $F_{(1,97)} = 7.19$, $p < 0.01$) but maintained elevated frequency relative to baseline (Figure 4C). *Onset* neuron firing rate also significantly increased (relative to the same baseline) to a peak of 10.7 ± 0.8 Hz ($F_{(1,123)} = 18.4$, $p < 0.001$) following presentation of 6 images in the Match phase, and declined rapidly to baseline firing level (1.5 ± 0.5 Hz) prior to occurrence of the behavioral response in the Match phase. The histograms below in Figure 4A-C show that the increase in *Onset* neuron firing that occurred during the trial was also synchronized to the pupil dilation events (rasters in Figure 4 A-C) that occurred on the same trials, and was thus correlated with image presentation in the different task phases (Sample vs. Match) as well as pupil dilation events that occurred on the same trials.

Figure 5 shows similar rasters and histograms for a *Sust.* FCx neuron in the Sample (Figure 5A), 3-Image Match (Figure 5B) and 6-Image Match phase (Figure 5C) of DMS trials. While the maximal pupil dilation frequency increased from 4.9 ± 0.6 Hz to 9.2 ± 0.8 Hz ($F_{(1,97)} = 14.40$, $p < 0.001$) as cognitive load increased from 1 to 6 images, the maximal firing rate of *Sust.* neurons decreased from 10.2 ± 0.8 Hz to 5.8 ± 0.5 Hz ($F_{(1,123)} = 11.76$, $p < 0.001$). Figure 5A-C shows that firing of *Sust.* FCx neurons was decoupled from dilation events, since *Sust.* neuron firing synchronized to pupil dilation decreased from 5.3 ± 0.4 Hz to 3.5 ± 0.3 Hz ($F_{(1,123)} = 11.20$, $p < 0.001$) from Sample to 6-Image Match. *Pre* and *Onset* FCx neurons (Figure 5D, left) showed increased synchronized firing rate per dilation event correlated as a function of increases in dilation frequency of (*Pre*: $r = 0.68$, $F_{(1,1579)} = 24.81$, $p < 0.001$; *Onset*: $r = 0.75$; $F_{(1,1732)} = 21.17$, $p < 0.001$). In contrast, *Sust.* and *Inhib.* neurons showed decreased synchronized firing rates as dilation frequency increased producing an inverse correlation (*Sust.*: $r = 0.83$, $F_{(1,1140)} = 19.09$, $p < 0.001$; *Inhib.*: $r = 0.61$, $F_{(1,1092)} = 20.41$, $p < 0.001$) with pupil dilation rate (Figure 5D, right).

Pupil Dilation and Cognitive Load

Figure 6A shows that performance in terms of mean % correct responses ($\pm$ SEM) significantly declined ($F_{(4,2948)} = 5.13$, $p < 0.001$) on trials with increased (distracter) images in the Match Phase (i.e. increased cognitive load [10]), and that this was associated with a significant increase in frequency (2 Image mean $\pm$ S.E.M. = 5.6 ± 0.2 Hz; 6 Image = 8.2 ± 0.4 Hz) of pupil dilation events for the same conditions ($F_{(6,8467)} = 4.21$, $p < 0.001$). Figure 6B shows a significant influence of number of images during the Match phase in terms of increasing the mean frequency of dilation for each of the 4 animals tested (asterisks * $p < 0.01$, ** $p < 0.001$ compared to Sample).

Pupil dilation frequency was also computed on probe trials (unfilled circles in Figure 6A) in which 6-8 images were presented randomly on the screen for 3 seconds (i.e. equivalent to a typical Match phase) within the session without either 1) a preceding Sample phase or 2) response contingent reward delivery. The frequency of pupil dilation on Probe trials was no different than that observed to single image presentation in the Sample Phase of the task ($F_{(1,8467)} = 2.01$, N.S.), indicating that the increase in dilation during the Match phase of the task was not related to the change in illumination produced by the increase in number of images. Figure 6A also shows no significant effect of frequency of saccades (circles: $F_{(6,8467)} = 1.03$, N.S.) as a function of increased numbers (2-6) of Match images during the task, and only a

slight increase on Probe trials (unfilled squares: $F_{(1,8467)} = 6.18$, $p < 0.01$). There was no significant effect on duration of eye movements (saccades) as a function of number of Match images ($F_{(6,8467)} = 2.75$, N.S.), and therefore saccade duration is not shown in Figure 6A. The result of this dual relationship between frequency of pupil dilation and neural firing with respect to cognitive load in the DMS task is shown in Figure 6C which verifies that firing of *Pre* and *Post* neurons increased significantly (*Pre*: $F_{(5,1932)} = 4.19$, $p < 0.001$; *Onset*: $F_{(5,1851)} = 4.42$, $p < 0.001$) as the number of Match phase images increased, while the firing rates of *Sust.* and *Inhib.* neurons were significantly decreased (*Sust.*: $F_{(5,1243)} = 4.54$, $p < 0.001$; *Inhib.*: $F_{(5,1150)} = 3.76$, $p < 0.001$) under the same conditions.

Pupil Dilation and Factors that Alter Cognitive Load

Figure 6 displays DMS performance and frequency of pupil dilation for three animals tested under different alertness conditions: 1) normal non-sleep deprived (control), 2) evening sessions just before light cycle change (“sleepy”) or 3) following a single night (30-36 hrs) of sleep deprivation (SD) [10,35]. A two-way ANOVA of number of images vs. alertness status (control, sleepy or SD) showed a significant effect on performance (Figure 6: DMS) as a function of 1) number of images in decreasing DMS performance ($F_{(4, 3271)} = 5.17$, $p < 0.001$), 2) state of alertness (sleepy and SD conditions) in reducing DMS performance ($F_{(2, 3271)} = 6.58$, $p < 0.001$), and 3) a significant interaction between number of images and state of alertness ($F_{(6, 3271)} = 3.98$, $p < 0.001$). A similar two-way ANOVA of frequency of pupil dilation revealed a significant effect of number of images with increased frequency of dilation (Figure 6, Pupil) on trials with more Match images ($F_{(5,3271)} = 4.83$, $p < 0.001$), and also as a function of alertness (sleepy or SD) conditions ($F_{(2,3271)} = 5.22$, $p < 0.01$). There was a significant interaction between number of images and alertness ($F_{(7,3271)} = 3.01$, $p < 0.01$) with respect to frequency of pupil dilation (Figure 7). Changes in dilation frequency did not result from changes in blink frequency during the Sample (mean Control: 0.55 ± 0.12 Hz, Sleepy: 0.51 ± 0.08 Hz, SD: 0.59 ± 0.15 Hz, $F_{(2,3271)} = 1.19$, N.S.) or Match (mean Control: 0.62 ± 0.11 Hz, Sleepy: 0.60 ± 0.15 Hz, SD: 0.66 ± 0.12 Hz, $F_{(2,3271)} = 1.47$, N.S.) phases. Consistent with results shown in Figure 6A, there was also no significant difference in saccade frequency (all $F_{(2,3271)} < 2.31$, N.S.) under the above conditions of variations in alertness.

Discussion

This report represents the first demonstration of changes in pupil dilation effected by cognitive workload in an NHP model as well as the first report of pupil dilation-sensitive FCx neuron activity related to the same changes in task related cognitive demand. These findings are in agreement with prior reports from this laboratory showing that NHP performance accuracy decreased on trials with increased number of images in the Match phase [10,16,35], substantiating the fact that cognitive workload regulates trial-by-trial performance in the DMS task. It has also been shown that sleep deprivation decreased DMS performance and speed of response [10,35], thereby effectively increasing cognitive workload in a manner similar to that demonstrated here as well as in humans [12].

In agreement with the above results, association between cognitive workload and pupil dilation is well established in humans [11,15,19,27,31,36,46,48]. The wavelet analysis used here is the same as described by Marshall et al., [27,29,50] in humans, which allowed specific identification of pupil dilations with rapid rise times (approx 100 ms) associated with recognizing or categorizing visual stimuli [11,27,46]. The employed analyses filtered-out noise in video recordings and slow dilation and constriction resulting from changing luminosity levels, as well as accounting for individual subject differences in absolute pupil diameter. In addition, the pupil dilation events were screened to remove artifacts from blinks and saccades. The frequency of onset of fast pupil dilations determined here in NHPs by wavelet analysis

exhibited increases in the exact same behavioral contexts (Sample and Match phases, Figures 2 & 4), under the exact same conditions of increased stimulus complexity and demand (Figure 5), as well as attentiveness during sleepiness and sleep deprivation (Figure 7), previously shown to be associated with increased cognitive load [10,35].

Neuronal discharges of FCx neurons synchronized to fast pupil dilations were classified into four unique patterns: *Pre*, *Onset*, *Sust.* or *Inhib.* which corresponded uniquely to the onset and time course of pupil dilation and constriction (Figure 3). *Pre* neurons fired immediately prior to onset of dilation, *Onset* cells discharged coincident with a rapid increase in dilation, while *Sust.* and *Inhib.* neurons decreased firing with dilation frequency consistent with maintenance of the pupil in the dilated state. This parceling of the time course of dilation suggests participation in a cortical network for feedback and control of pupil dilation by FCx neurons as proposed by Einhauser et al. [11].

Pre and *Onset* neurons also showed firing rate increases during Sample and Match phases due to increased frequency of pupil dilation events during those same phases. This suggests as previously proposed [2,37,43,44], that rather than encoding specific “static” visual features of the images [16], FCx neurons encode information regarding the “processing” of those features as necessary for effective task performance. Lee and Keller [23] showed that if more than one stimulus location was possible within a trial, FCx neural firing was altered from when the stimulus position was fixed with some cells increasing and others decreasing firing rates. Although pupil dilation was not measured in that study, the correlation of *Pre*, *Onset*, *Sust.* or *Inhib.* cell firing with both pupil dilation and increasing number of Match images in the current study, suggests that the link between FCx neuron firing and cognitive workload, may be pupil dilation frequency and its role in image processing.

Rutishauser and Koch [38] propose a neural network model of visual search based on feature extraction by prefrontal neurons. In their model, firing correlates of FCx neurons such as those found here would provide critical indicators of when particular features were encountered, and would provide a direct correlate of cognitive load based on the frequency and duration with which the visual features of a scene were searched. Moreover, Einhauser et al. [11] suggest that feature recognition results in activation of neurons in locus coeruleus (LC) [1], which in turn produces pupil dilation via direct and/or indirect noradrenergic projections to muscles in the iris [9]. It has also been shown by Moresi et al. [31] that fast pupil dilation precedes response selection, therefore frequency of pupil dilation in this context could provide a continuous monitor of visual searching and feature recognition [5,22].

We propose that the increase in frequency of fast pupil dilation events on high cognitive load DMS trials with increased numbers of nonmatch or distracter images (Figures 4-7), reflects increased processing to recognize or detect specific feature elements of the Sample image in the visual field during the Match phase of the task. The synchronized activation of FCx neurons, in conjunction with locus coeruleus (LC) could thereby confirm that a component of the visual target has been detected [1]. This model of pupil dilation guided search is portrayed in Figure 8. The diagram illustrates that subsequent LC activation to produce corresponding pupil dilation [11] could act as feedback to frontal cortical eye movement neural circuitry to direct the search for other relevant features [2,4,7,22]. In the proposed model the two types of FCx neurons serve opposite roles to account for changes in pupil dilation frequency that interacts with factors that regulate search modes to adjust to changes in the degree of cognitive demand (Figure 8, Target). Immediate target detection and recognition are clearly at the opposite end of the continuum from visual tasks requiring visual search strategies to handle Target complexity (Figure 8, Cognitive Load). Therefore a tight coupling between information gained from the target on a given dilation event and what to look for next requires processing and guidance by either of the two FCx systems depicted in Figure 8. The same information could

be transmitted to other cortical and subcortical areas, such as prefrontal cortex [14], hippocampus and the medial temporal lobe [16,37], and striatum [40], previously shown to be critical for cognitive processing in the DMS task. Therefore increased frequency of pupil dilation may not only reset eye movements more efficiently [11], but may also serve to refine the visual search [4,38], when viewing complex scenes under conditions of high cognitive workload. More importantly, however is the possibility that fast pupil dilations may indicate critical detection in planned processing stages. FCx neuron firing would therefore not only provide feedback for directing visual search, but also provide a means of screening visual features for those most salient to task demands (Cognitive inputs, Figure 8). Such feedback and filtering could thus be utilized to reduce interference by non-task-related features, resulting in improved performance under conditions of high cognitive load.

Several alternative interpretations are possible, such as attention or arousal, for both the alteration in frequency of pupil dilation and the observed neuronal correlates reported here. However, the wavelet analysis was selected specifically to control for pupil dilation and contraction > 500 ms that have been shown to be associated with changes in attention [27]. Pupil dilation on probe trials (Figure 6A) did not increase in frequency even when visual complexity (# of Match phase images) increased. While neuronal responses in the FEF have been primarily associated with saccades [39,51], neurons in SEF have been shown to correlate with eye and limb movements [13,42]. In the current study, the influence of limb movements was not a factor, since identical hand/arm movements occurred on both high and low cognitive load trials in which differences in frequency of pupil dilation were observed. However, with respect to eye movements, the demonstration here of a specific correlation of FCx cell firing with onset of fast pupil dilations is consistent with the role of SEF neurons in selection of saccades during visual search [33].

The results shown here demonstrate application of a noninvasive measure of cognitive workload; frequency of fast pupil dilation, to an NHP model in which firing from FCx neurons revealed correlations with cognitive workload-sensitive dilation frequency (Figure 5) in a fully characterized cognitive task. These results provide important new insight into the neural linkages for this well-established observation in humans [3,28,41,45,46,48,49]. The ability to record single neuron activity correlated with cognitive workload and pupillary dilation has provided increased understanding of the functional basis of the pupil dilation signal within the brain (Figure 8) and will facilitate future evaluation of noninvasive pupillometric measures in humans.

Acknowledgments

The authors acknowledge the able assistance of Michael Todd, Joshua Long, Joseph Noto, Kathryn Gill, and Joel L. Davis. Support for this work was provided by ONR N00014-06-1-0397, DARPA N66001-09-C-2080, NSF EEC 0310723, NIH DA06634, DA023573, to S.A.D and DA08549 to R.E.H.

References

1. Aston-Jones G, Cohen JD. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu Rev Neurosci* 2005;28:403–450. [PubMed: 16022602]
2. Bayerl P, Neumann H. A neural model of feature attention in motion perception. *Biosystems* 2007;89:208–215. [PubMed: 17280774]
3. Beatty J. Task-evoked pupillary responses, processing load, and the structure of processing resources. *Psychol Bull* 1982;91:276–292. [PubMed: 7071262]
4. Bichot NP, Chenthal RS, Schall JD. Continuous processing in macaque frontal cortex during visual search. *Neuropsychologia* 2001;39:972–982. [PubMed: 11516449]
5. Bichot NP, Schall JD. Effects of similarity and history on neural mechanisms of visual selection. *Nat Neurosci* 1999;2:549–554. [PubMed: 10448220]

6. Bruce CJ, Goldberg ME, Bushnell MC, Stanton GB. Primate frontal eye fields. II. Physiological and anatomical correlates of electrically evoked eye movements. *J Neurophysiol* 1985;54:714–734. [PubMed: 4045546]
7. Cariani PA. Temporal codes and computations for sensory representation and scene analysis. *IEEE Trans Neural Netw* 2004;15:1100–1111. [PubMed: 15484887]
8. Carpenter PA, Just MA, Reichle ED. Working memory and executive function: evidence from neuroimaging. *Curr Opin Neurobiol* 2000;10:195–199. [PubMed: 10753796]
9. de Seze J, Arndt C, Stojkovic T, Ayachi M, Gauvrit JY, Bughin M, Saint MT, Pruvo JP, Hache JC, Vermersch P. Pupillary disturbances in multiple sclerosis: correlation with MRI findings. *J Neurol Sci* 2001;188:37–41. [PubMed: 11489283]
10. Deadwyler SA, Porrino L, Siegel JM, Hampson RE. Systemic and nasal delivery of orexin-A (Hypocretin-1) reduces the effects of sleep deprivation on cognitive performance in nonhuman primates. *J Neurosci* 2007;27:14239–14247. [PubMed: 18160631]
11. Einhauser W, Stout J, Koch C, Carter O. Pupil dilation reflects perceptual selection and predicts subsequent stability in perceptual rivalry. *Proc Natl Acad Sci U S A* 2008;105:1704–1709. [PubMed: 18250340]
12. Franzen PL, Siegle GJ, Buysse DJ. Relationships between affect, vigilance, and sleepiness following sleep deprivation. *J Sleep Res* 2008;17:34–41. [PubMed: 18275553]
13. Fujii N, Mushiaki H, Tanji J. Distribution of eye- and arm-movement-related neuronal activity in the SEF and in the SMA and Pre-SMA of monkeys. *J Neurophysiol* 2002;87:2158–2166. [PubMed: 11929933]
14. Funahashi S. Prefrontal cortex and working memory processes. *Neuroscience* 2006;139:251–261. [PubMed: 16325345]
15. Granholm E, Asarnow RF, Sarkin AJ, Dykes KL. Pupillary responses index cognitive resource limitations. *Psychophysiology* 1996;33:457–461. [PubMed: 8753946]
16. Hampson RE, Pons TP, Stanford TR, Deadwyler SA. Categorization in the monkey hippocampus: A possible mechanism for encoding information into memory. *Proc Natl Acad Sci U S A* 2004;101:3184–3189. [PubMed: 14978264]
17. Hasegawa M, Carpenter PA, Just MA. An fMRI study of bilingual sentence comprehension and workload. *Neuroimage* 2002;15:647–660. [PubMed: 11848708]
18. Hayhoe M, Ballard D. Eye movements in natural behavior. *Trends Cogn Sci* 2005;9:188–194. [PubMed: 15808501]
19. Hyona J, Tammola J, Alaja AM. Pupil dilation as a measure of processing load in simultaneous interpretation and other language tasks. *Q J Exp Psychol A* 1995;48:598–612. [PubMed: 7568993]
20. Just MA, Carpenter PA. The intensity dimension of thought: pupillometric indices of sentence processing. *Can J Exp Psychol* 1993;47:310–339. [PubMed: 8364533]
21. Kenemans JL, Verbaten MN, Melis CJ, Slangen JL. Visual stimulus change and the orienting reaction: event-related potential evidence for a two-stage process. *Biol Psychol* 1992;33:97–114. [PubMed: 1525300]
22. Lanyon LJ, Denham SL. A model of active visual search with object-based attention guiding scan paths. *Neural Netw* 2004;17:873–897. [PubMed: 15288904]
23. Lee KM, Keller EL. Neural activity in the frontal eye fields modulated by the number of alternatives in target choice. *J Neurosci* 2008;28:2242–2251. [PubMed: 18305257]
24. Lipp OV, Neumann DL. Attentional blink reflex modulation in a continuous performance task is modality specific. *Psychophysiology* 2004;41:417–425. [PubMed: 15102127]
25. Lipp OV, Neumann DL, Pretorius NR, McHugh MJ. Attentional blink modulation during sustained and after discrete lead stimuli presented in three sensory modalities. *Psychophysiology* 2003;40:285–290. [PubMed: 12820869]
26. Marshall, SP. Assessing cognitive engagement and cognitive state from eye metrics. In: Schmorow, DD., editor. *Foundations of Augmented Cognition*, Las Vegas, NV, Jul 22-27-2005. Mahwah, NJ: Lawrence Erlbaum Associates; 2005. p. 312-320.
27. Marshall SP. Identifying cognitive state from eye metrics. *Aviat Space Environ Med* 2007;78:B165–B175. [PubMed: 17547317]

28. Marshall, SP. Measures of attention and cognitive effort in tactical decision making. In: Cook, M.; Noyes, J.; Masakowski, Y., editors. *Decision Making in Complex Environments*. Aldershot, Hampshire, UK: Ashgate Publishing; 2007. p. 321-332.
29. Marshall, SP.; Davis, CL.; Knust, SR. The index of cognitive activity: Estimating cognitive effort from pupil dilation. San Diego, CA: Eyetracking, Inc; 2005.
30. Melis C, van Boxtel A. Differences in autonomic physiological responses between good and poor inductive reasoners. *Biol Psychol* 2001;58:121-146. [PubMed: 11600241]
31. Moresi S, Adam JJ, Rijcken J, Van Gerven PW, Kuipers H, Jolles J. Pupil dilation in response preparation. *Int J Psychophysiol* 2008;67:124-130. [PubMed: 18067982]
32. Neumann DL, Lipp OV. Spontaneous and reflexive eye activity measures of mental workload. *Australian Journal of Psychology* 2002;54:174-179.
33. Parton A, Nachev P, Hodgson TL, Mort D, Thomas D, Ordidge R, Morgan PS, Jackson S, Rees G, Husain M. Role of the human supplementary eye field in the control of saccadic eye movements. *Neuropsychologia* 2007;45:997-1008. [PubMed: 17069864]
34. Paxinos, G.; Huang, XF.; Toga, AW. *The Rhesus Monkey Brain in Stereotaxic Coordinates*. San Diego, CA: Academic Press; 2003.
35. Porrino LJ, Daunais JB, Rogers GA, Hampson RE, Deadwyler SA. Facilitation of task performance and removal of the effects of sleep deprivation by an amphetamine (CX717) in nonhuman primates. *PLoS Biol* 2005;3:e299. [PubMed: 16104830]
36. Porter G, Troscianko T, Gilchrist ID. Effort during visual search and counting: insights from pupillometry. *Q J Exp Psychol (Colchester)* 2007;60:211-229. [PubMed: 17455055]
37. Rolls ET, Deco G. Attention in natural scenes: Neurophysiological and computational bases. *Neural Netw* 2006;19:1383-1394. [PubMed: 17011749]
38. Rutishauser U, Koch C. Probabilistic modeling of eye movement data during conjunction search via feature-based attention. *J Vis* 2007;7:5. [PubMed: 17685788]
39. Schall JD. Neuronal activity related to visually guided saccadic eye movements in the supplementary motor area of rhesus monkeys. *J Neurophysiol* 1991;66:530-558. [PubMed: 1774585]
40. Sil'kis IG. The contribution of synaptic plasticity in the basal ganglia to the processing of visual information. *Neurosci Behav Physiol* 2007;37:779-790. [PubMed: 17922242]
41. St John M, Kobus D, Morrison J, Schmorow D. Overview of the DARPA Augmented Cognition Technical Integration Experiment. *Int J Hum Comput Interact* 2004;17:131-149.
42. Tanji J. The supplementary motor area in the cerebral cortex. *Neurosci Res* 1994;19:251-268. [PubMed: 8058203]
43. Treue S, Martinez-Trujillo JC. Attending to features inside and outside the spotlight of attention. *Neuron* 2007;55:174-176. [PubMed: 17640519]
44. Tyler LK, Stamatakis EA, Bright P, Acres K, Abdallah S, Rodd JM, Moss HE. Processing objects at different levels of specificity. *J Cogn Neurosci* 2004;16:351-362. [PubMed: 15072671]
45. van der Meer E, Friedrich M, Nuthmann A, Stelzel C, Kuchinke L. Picture-word matching: flexibility in conceptual memory and pupillary responses. *Psychophysiology* 2003;40:904-913. [PubMed: 14986843]
46. Van Gerven PW, Paas F, Van Merriënboer JJ, Schmidt HG. Memory load and the cognitive pupillary response in aging. *Psychophysiology* 2004;41:167-174. [PubMed: 15032982]
47. Van Orden KF, Jung TP, Makeig S. Combined eye activity measures accurately estimate changes in sustained visual task performance. *Biol Psychol* 2000;52:221-240. [PubMed: 10725565]
48. Van Orden KF, Limbert W, Makeig S, Jung TP. Eye activity correlates of workload during a visuospatial memory task. *Hum Factors* 2001;43:111-121. [PubMed: 11474756]
49. Verney SP, Granholm E, Marshall SP. Pupillary responses on the visual backward masking task reflect general cognitive ability. *Int J Psychophysiol* 2004;52:23-36. [PubMed: 15003370]
50. Verney SP, Granholm E, Marshall SP, Malcarne VL, Saccuzzo DP. Culture-fair cognitive ability assessment: information processing and psychophysiological approaches. *Assessment* 2005;12:303-319. [PubMed: 16123251]

51. Yamamoto J, Ikeda A, Satow T, Matsuhashi M, Baba K, Yamane F, Miyamoto S, Mihara T, Hori T, Taki W, Hashimoto N, Shibasaki H. Human eye fields in the frontal lobe as studied by epicortical recording of movement-related cortical potentials. *Brain* 2004;127:873–887. [PubMed: 14960503]

Figure 1.

Pupillometry and detection of fast pupil dilation events. **A:** Nonhuman primate (NHP) subjects ($n=4$) performed a visual Delayed-Match-to-Sample (DMS) task while pupil diameter was continuously recorded via an EyeLink 1000 system. Diagram shows a subject performing the DMS task by viewing the computer projection screen (see below) through a 45° mirror that reflected only infra-red (IR) images of the eye to an IR-sensitive camera. EyeLink software recorded position and diameter of the pupil, movement (saccade) or eye blinks for removal of artifact from the pupil diameter record (Inset). **Sample:** Represents *Sample phase* of DMS task in which a single image is displayed. **Match:** Represents *Match phase* DMS task in which the same (Sample image is shown along with 1-7 additional irrelevant (i.e. nonmatch) images. [10,35] **B:** Continuous traces of eye position, pupil diameter and blink/saccade status. Top: Blue and green traces illustrate continuous (5 sec) record of eye position based on iris/corneal reflectivity measured by the EyeLink 1000 system. Position is recorded in pixels of the video image; each pixel represents approx 1/15 degree of arc change in gaze position. Middle: Red trace indicates pupil diameter for the same time interval shown above. Diameter is plotted in pixels of video image, each pixel represents approximately 1 μ m. Bottom: Blink/saccade status in terms of movement (saccade), fixation (Fix) or eyelid blink (Blink) on the same time scale as location and diameter above. Shaded regions extended upward from blinks (pink shading) and saccades (gray shading) to indicate portions of the video record that may induce artifacts in pupil diameter measurement, and are thus excluded from further analysis. **C:** Wavelet decomposition of pupil diameter. Top trace depicts 400 sec segment of 1000 Hz pupil diameter recording. Pupil diameter is measured in pixels relative to the video image of the iris and pupil and ranged from 8,000-11,000 pixels. Middle and bottom traces show results of a wavelet decomposition (see Methods), yielding level 1 approximation (middle trace) and details (bottom trace) corresponding to low frequency (slower than 100 ms) and high frequency (faster than 100 ms), respectively, components of pupil diameter fluctuations. Dots indicate time stamped pupil dilation events corresponding to the onset of fast pupil dilation that have been derived by applying a threshold filter to the high frequency component of the wavelet decomposition. **D:** Expansion of a 1250 ms section of pupil diameter (black trace), wavelet

approximation (red trace), and wavelet details (blue trace) showing fast pupil diameter changes. Arrows: dilation events representing greater than 10% increase in pupil diameter.

Figure 2.

Frontal cortical activity associated with pupil dilation. **A:** Recording location in Frontal Cortex (FCx). Top: Anterior lateral view of rhesus brain. Purple dot indicates recording electrode location allowing access to Brodmann Areas 6 & 8, overlying the Frontal Eye Fields. Bottom: Electrodes were positioned antero-medial to arcuate sulcus, at depths of 1-5 mm below surface of brain to record neurons in Layers 2 and 5 of FCx. **B:** Illustration of neural firing with respect to pupil dilation. Superimposed 1.0 sec traces showing rapid changes in pupil diameter (pixel units, see scale bar: 500 cm = approx. 0.15 cm). Traces are synchronized to the onset of wavelet-detected fast pupil dilation events (vertical dashed line). Rastergram shows individual cell spikes (dots) recorded from a single FCx neuron synchronized to individual pupil dilations (vertical dashed line) for 100 successive dilation events (rows). Perievent histogram (PEH, below) shows mean firing rate averaged across individual dilation events shown in raster. Inset: superimposed examples of FCx cell waveform. Asterisks (** $p < 0.001$): significant increase in neural firing rate (0-250 ms post dilation onset) compared to baseline (-250 to 0 ms). **C:** Pupil dilation and neuronal cell firing in Sample and Match phase of DMS trial. The time line demonstrates phases of a DMS trial. Rastergrams and PEHs illustrate single neural firing and

dilation events on same time scale (0-2.0s) in individual DMS trials commencing at stimulus presentation and extending to time of behavioral response (Response). PEHs reflect mean rates of occurrence across trials (bars = 50ms bins) for both wavelet-derived pupil dilation events in dilations/sec (upper) and recorded spikes from FCx neurons (lower). Trials (n=50) are shown for Sample and Match phases of DMS task within a single session. Dashed white line illustrates decreased frequency of fast pupil dilation over Match Phase following initial stimulus presentation. Abbr: intertrial interval (ITI); Sample phase; Match phase; juice reinforcement (Reinf.).

Figure 3.

Differential firing of FCx neurons in relation to onset of pupil dilation. **A-B:** Transient firing peaks synchronized to the *onset* of pupil dilation. **A:** *Pre*-dilation FCx neurons (n=28 cells) exhibit firing increases immediately preceding onset of pupil dilation shown as dashed vertical line (0 sec). Top graph: traces show mean firing rate of *Pre*-dilation FCx neurons in relation to individual pupil dilation events within a single behavioral session (n=500-1000 dilation events per session). Individual cell firing rate traces are constructed with the same resolution as the PEH below ± 0.5 sec synchronized to the dilation events (time = 0s) with 100 ms bins which represents mean ($\pm$ S.E.M.) firing rate across all *Pre*-dilation FCx neurons (n=28). Brackets indicate baseline (-500 to -250 ms) and peak regions (-150 to 0 ms) compared statistically. Asterisks (**p<0.001) indicate significant increase vs. baseline rate. **B:** *Dilation Onset* FCx neurons (n=24) show a brief firing increase coincident with onset of pupil dilation and a secondary peak at 200 ms. Traces (top) and histogram (below) represent individual cell firing and the means across cells respectively as in *Pre*. Post dilation peak firing (0 to 50 s) differed markedly (**p<0.001) from baseline firing but the secondary peak did not. **C:** FCx neurons (n=17) showing longer duration, *Sustained* (*Sust.*), significant increases in firing (**p<0.001) following onset of the pupil dilation which was maintained for 500-700 ms before returning to baseline firing rate (500 ms shown). Traces (individual neuron firing rates) and histograms (mean across neurons) constructed as in A. **D:** *Inhibited* (*Inhib.*) FCx neurons

(n=17) exhibited significantly decreased (**p<0.001) firing immediately following onset of pupil dilation for approximately 250 ms at which point firing returned to baseline levels. Traces (individual neuron firing rates) and histograms (mean across neurons) constructed as in A. **E**: PEHs (colored lines) of each of the four patterns of dilation-synchronized types of FCx neuron firing (A-D) are shown plotted with the mean pupil diameter (black lines) changes recorded before and after (± 0.5 s) the dilation events (vertical dashed line) used to construct the respective firing patterns of each cell type. Colored lines represent outline of PEHs for each of FCx neuron type in A-D.

Pupil Dilation and *Onset* Neural Firing in DMS Task

Figure 4.

Pupil dilation events and FCx neural firing during Sample and Match image presentations in DMS task. Rastergrams and PEHs at top show firing of a single *Onset* (Figure 3) FCx neuron on DMS trials in response to images presented (0s, vertical solid line) in the Sample (A) or Match (3-Images, B; 6-images, C) phases of task. Dashed horizontal line indicates the same frequency on PEHs for comparison across number of images in Sample and Match phases and indicates increased *Onset* FCx neuron firing is coincident with the increased frequency of pupil dilation events (above) during Match image presentation. Single PEHs at bottom of Figure depict firing of the same *Onset* FCx neuron synchronized to the pupil dilation events (vertical line, Dilation) shown in the respective rastergrams for the same trials in each of the DMS stimulus presentation conditions (Sample and Match 3&6 images). Asterisks indicate significant increases in firing rate (** $p < 0.001$) compared to Sample image increase.

Pupil Dilation and Sust. Neural Firing in DMS Task

Figure 5.

Pupil dilation events and FCx neural firing for a single *Sust.* FCx neuron. Rastergrams and PEHs as in Figure 4 depict frequency of pupil dilation events and neural firing in response to images presented in Sample (A) or Match for 3-Images (B) or 6-images (C). Dashed horizontal lines refer to dilation and firing frequencies to Sample image presentation. Although frequency of pupil dilations increased as the number of images increased (Pupil Dilation, PEHs), *Sust.* FCx neuron firing rate decreased on the same trials (Neural firing). PEHs in bottom row depict “dilation triggered” firing of the same *Sust.* neuron synchronized to the onset of the pupil dilations shown rastergrams (Figure 4). Comparison of dotted horizontal line in C indicates decrease in neural firing triggered by dilation events on 6-image trials. Asterisks indicate significant (** $p < 0.001$) changes (increases or decreases) compared to presentation of Sample image. **D:** Relationship of the 4 categories of FCx neurons to frequency of pupil dilation events across all DMS trials. Left: *Pre* and *Onset* FCx neurons (Figure 2 A&B). As the frequency of pupil dilation increased synchronized firing rate per dilation event increased also (positive correlation). Dashed lines represent regression and correlation for *Pre* cell type firing rates (slope = 1.11, $r = 0.68$); dashed-dotted line shows regression and correlation for *Onset* cell type firing rates (slope = 0.62, $r = 0.75$) with respect to frequency of dilation. Right: *Sust.* and *Inhib.* Neurons. As dilation frequency increased with respect to increased # of images in Match phase of the DMS task (A-C above) these cell types (Figure 2 C&D) showed decreased firing rates. Dashed line – *Sust.*: slope = -0.45, $r = 0.83$; Dashed-dotted line – *Inhib.*: slope = -1.05, $r = 0.61$.

Figure 6.

Pupil dilation changes related to differences in cognitive load during performance of DMS task. **A:** Relationship between frequency of pupil dilation events (filled circles) and DMS performance is shown as mean ($\pm$ S.E.M.) frequency (dilations per second) computed for the Sample (1 image) and the Match phase for 2-6 image trials. Asterisks indicate significant (** $p < 0.001$) increases in frequency of dilation events for 2-6 image trials compared to Sample image frequency for the same animals on trials with 2-6 images in Match phase (DMS performance). Performance on trials with 3-6 images was significantly lower than trials with only 2 images in the Match phase ($\dagger p < 0.001$). Frequency of eye movements (saccades, filled squares) are plotted across the same DMS events and showed no significant change. Probe trials consisted of 6 images projected on the screen for 2 sec without a preceding Sample image, and no response requirement. Frequency of pupil dilation (unfilled circle) or saccades (unfilled square) is shown for comparison with dilation frequency on 6-image trials (filled circles). **B:** Pupil dilation frequency vs. number of images for each individual animal in DMS task. Bars represent mean ($\pm$ S.E.M.) frequency of pupil dilation sorted by Sample (1 Image) and Match (2-6 Images) phases. * $p < 0.01$, ** $p < 0.001$ reflect significant increases in frequency of dilations in Match phase for 2-6 image trials vs. Sample phase on the same trials (NHP #1: $F_{(6,2502)} = 3.74$, $p < 0.001$; NHP #2: $F_{(6,2381)} = 3.57$, $p < 0.001$; NHP #3: $F_{(6,1723)} = 3.29$, $p < 0.001$; NHP #4: $F_{(6,1891)} = 3.41$). **C:** Effect of varying cognitive load (different number of Match phase images) on firing of the 4 different types of FCx neurons shown in Figures 2. Mean ($\pm$ S.E.M.) peak firing rates *Pre*, *Onset*, *Sust.* FCx neurons shown sorted by number of images on DMS trials (Sample – 1 image; Match – 2-6 images). For *Inhib.* FCx neurons the minimum firing rate within 0 to 500 ms following image presentation was plotted for Sample and Match phases

over the same trials. Asterisks (* $p < 0.01$, ** $p < 0.001$) indicate significant increases or decreases from firing in Sample phase (1 img).

Manipulation of Cognitive Load

Figure 7.

Effect of increased stress on cognitive performance in DMS task. Sleep manipulations increase cognitive demand (workload) and neuronal activation in Prefrontal Cortex (refs) during DMS task performance in 3 NHPs. Changes in mean ($\pm$ S.E.M) frequency of pupil dilation (filled symbols, solid lines) and DMS performance (unfilled symbols, dashed lines) assessed following sleepy (tested in task 6 hours later than normal; triangles) and sleep deprived (30-32 hrs of total sleep deprivation; squares) testing conditions. Asterisks (*p<0.01, **p<0.001) indicate significant differences from normal testing (non-sleep manipulated) sessions.

Model of Pupil Dilation and Guided Search

Figure 8.

Proposed model of pupil dilation and FCx neuron firing during task-directed visual search strategies under different degrees of cognitive workload adapted from Rutishauser et al. [38] and Einhauser et al. [11]. The model shows that during visual search, the locus coeruleus is activated and the pupil dilates when a visual element of the target is detected. Pupil dilation frequency is a function of the degree of cognitive load and increases in order to increment the search for related elements if the target load is substantial (High), or is reduced when load is minimal (Low) as signified by target recognition. The current model proposes a neural substrate containing *Sust.* and *Inhib.* neurons “tuned” for low cognitive load trials (Low) in which few elements trigger prolonged pupil dilations with a subsequent shift to different targets signified by object recognition. High cognitive load trials (High) activate *Pre* and *Onset* neurons tuned for detection of increased numbers of elements or features of target images required for eventual detection and recognition. Such activation also decreases firing in *Sust.* and *Inhib.* Neurons (“suppress”) as a function of increased frequency of pupil dilation. Each of the neural populations in turn activates the locus coeruleus which modulates pupil dilation and feeds back to each type of FCx neuron to either: 1) further guide search and detection (Continue Search) with increased dilation frequency (*Pre* and *Onset* neurons) or 2) confirm target features by decreasing frequency and increasing dilation duration (*Sust.* and *Inhib.* neurons). Finally, visual search is guided by “cognitive inputs” from; 1) frontal cortex (FCx) for extraction of key visual features [4], 2) hippocampus for memory and categorization of image [16], 3) striatum for salience of cues and reinforcement [40]; and 4) medial temporal lobe for recognition and extraction of the target features [8,16].