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Fentanyl, an agonist at the mu opioid receptor, depresses pupillary unrest☆

Michael P. Bokoch [a], Matthias Behrends [a], Andrew Neice [b], Merlin D. Larson [a,*]

[a] Department of Anesthesia and Perioperative Care, University of California, San Francisco, 94143, United States
[b] Department of Anesthesia and Perioperative Medicine, Oregon Health and Science University, Portland, OR 97239, United States

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ABSTRACT

Pupillary unrest is a chaotic fluctuation in pupil size that is observed in darkness with the onset of drowsiness, and in ambient light. The mechanism of pupillary unrest in darkness as well as in ambient light is unknown but studies suggest that it is caused by fluctuating activity in the Edinger–Westphal (E.W.) nucleus. Neurons in the periaqueductal gray with oscillating firing patterns that are inhibitory to the E.W. nucleus have been described in the cat. We theorized that such oscillating neurons produce pupillary unrest in light and would be depressed by agents, such as opioids, known to depress inhibitory pathways in the midbrain. An infrared pupillometer was used to measure the effect of light on pupillary unrest in eight volunteer subjects, and on 20 patients scheduled for knee arthroscopy who received fentanyl as premedication. Pupillary unrest was quantified through spectral analysis of fast Fourier transforms. Sixteen-second measurements of pupil size at 33 Hz were filtered to eliminate blink artifacts and baseline drift. Pupillary unrest was augmented by excitation of the E.W. nucleus by light and was depressed by 40 ± 20% after the administration of the moderate dose of 1 mcg/kg of fentanyl. Recovery from the drug effect was observed. Based upon the data from this study we propose that pupillary unrest in light originates within oscillating inhibitory neurons that intermittently depress the E. W. nucleus.

1. Introduction

Pupillary unrest in the presence of ambient light (PUAL) is a chaotic fluctuation in the size of the pupil (Movie 1) that varies with light intensity (Stark, 1969; Warga et al., 2009; Loewenfeld, 1999). It has been noted to be equal in both eyes, is not dependent on the sympathetic innervation of the dilator muscle of the iris, is not produced by intermittent attempts to focus on near objects (Hunter et al., 2000; Loewenfeld, 1999), and is not the result of an unstable feedback servomechanism (Stark and Cornsweet, 1958).

The mechanism of how PUAL is produced is unknown but one theory is that it results from an interaction between excitatory and inhibitory inputs into the neurons of the Edinger–Westphal (E.W.) nucleus (also called the pupilloconstrictor nucleus) that controls the tension in the sphincter muscle of the iris (Loewenfeld, 1999). E.W. neurons are spontaneously active pacemaker cells that, when devoid of synaptic input, have a high intrinsic firing rate, resulting in pupillary constriction (Ichinohe and Shoumura, 2001). Inhibition of the E.W. nucleus consequently results in pupillary dilation.

We hypothesized that intermittent changes in the inhibition of the E.W. nucleus are responsible for the pupillary oscillations seen in PUAL. It has been demonstrated that short axon neurons with bursting firing patterns are present in the feline periaqueductal gray matter (PAG) that are inhibitory to the E.W. nucleus (Smith et al., 1968). These dilation-correlated neurons produce pupillary dilation when stimulated electrically (Smith et al., 1968). PUAL could be caused by fluctuating activity of these dilation-correlated neurons or similar oscillating neurons that indirectly alter pupil size. In order to test this hypothesis it would be necessary to depress the functional effect of these inhibitory neurons.

While the exact mechanism of opioid induced miosis is not completely known, there is evidence that opioids increase the activity of the E.W. nucleus by exerting a depressant effect on inhibitory neurons that project into this nucleus (Larson, 2008). Such disinhibition results in miosis, due to the high intrinsic firing rate of the E.W. neurons (Ichinohe and Shoumura, 2001). If the central inhibitory effects of opioids extend to dilation-correlated neurons or similar oscillating neurons that inhibit the E.W. nucleus, the administration of opioids would also suppress PUAL.

The present study investigates the effects of a moderate dose of an opioid on PUAL. Suppression of PUAL by opioid administration would support the hypothesis that fluctuating inhibition of the E.W. nucleus

☆ Received from the Department of Anesthesiology and Perioperative Care, University of California, San Francisco, CA 94143-0648, United States.

* Corresponding author at: Department of Anesthesia and Perioperative Care, University of California, San Francisco, CA 94143-0648, United States. Tel.: +1 415 885 7412; fax: +1 415 476 9516.
E-mail address: larsonm@anesthesia.ucsf.edu (M.D. Larson).

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causes PUAL. Furthermore if a dose-dependent depression of PUAL by opioids could be established, measuring changes of PUAL could have clinical value, as the suppression of PUAL may be an indicator for the central effects of opioids.

2. Materials and methods

2.1. Study subjects

After institutional approval and informed consent, we studied 20 healthy (ASA 1 – 2) patients undergoing arthroscopic procedures on the knee and 8 healthy volunteers. We excluded patients taking medications known to alter pupil size or pupillary reflexes.

2.2. Calibration and testing of infrared pupillometer

Measurement of PUAL was performed with a modified commercial portable infrared pupillometer (Model RD—NL013, Neuroptics, Irvine, California) (Taylor et al., 2003; Du et al., 2005). A constant source of diffuse blue light with an intensity of 100 lx and a wavelength of 460 nm, covering 20° of the visual field, was directed into the left pupil. The light source was 2.5 cm from the corneal surface and patients were informed to look directly into this opaque blue disc with the left eye. Ambient light was excluded from the right eye with a rubber cup projecting from the lens of the pupillometer. Pupil size from the right eye was measured for 16 s, by measuring the reflection of infrared light. Recordings were taken at a frequency of 33 Hz (0.03 s between sample times). The average pupil diameter over the 16-second measurement was calculated and reported as pupil size for that measurement.

Measurements taken with infrared pupillometry are prone to artifacts that arise from blinks, partial lid closures, and eye movements. We modified the computerized methods as described by McLaren et al. (1992), Merritt et al. (1994) and Ludtke et al. (1998) to remove artifacts that did not arise from actual movements of the pupil. Following artifact removal from the raw data with our algorithm, we used fast Fourier transforms (FFTs) to analyze the frequency components of each measurement. We analyzed the frequency components of the pupil size/time graphs up to 16 Hz, a frequency that exceeds the ability of the smooth muscles of the iris to follow a rapid intermittent light stimulus (Stark, 1963). Our measure of PUAL was the sum of amplitudes across specific frequencies.

Prior to initiating the fentanyl drug study, we sought to confirm the reliability of the portable infrared pupillometer. Calibration for movement artifacts and accuracy of the measurements was performed on metal holes of known diameter. We studied metal holes having known calibrated diameters of 2.6, 3.6 and 4.8 mm. The metal hole was moved during recording to assess the ability of the pupillometer to track the aperture and provide reliable readings during spontaneous eye movements. FFTs of these measurements established the noise and baseline stability of the pupillometer.

Further preliminary experiments were performed on eight consenting volunteer subjects to establish that pupillary unrest when measured with a portable instrument was dependent upon light intensity, as has been previously described using desk top pupillometers (Warga et al., 2009). To perform this preliminary study, we placed the blue light source in front of the non-measured (left) eye in a dark room. The light was then turned off and on to assess the effect on pupillary unrest. We then varied the intensity of light and measured both pupil size and PUAL.

2.3. Study of opioid effect on PUAL

Patients in the drug study were admitted to a preoperative holding area with ambient lighting of approximately 75 lx. Two measurements of PUAL were taken separated by 4–6 min. Following these baseline measurements, intravenous fentanyl 1 mcg/kg was administered and a repeat measurement of PUAL was taken 4–5 min later. After a variable delay of up to 45 min, patients were taken into the operating room where arthroscopy of the knee was performed. General anesthesia was provided during the procedure with a combination of midazolam, propofol, and sevoflurane. To assess recovery from fentanyl, measurements of PUAL were taken from selected patients in whom induction of general anesthesia was delayed more than 15 min from the time of fentanyl administration. In some cases this delay resulted from the placement of a femoral nerve block; a prior study (unpublished) had determined that this procedure had no effect on PUAL.

PUAL was quantified by calculating the area under the curves (AUC) of the Fourier transforms of pupil diameter. Specifically, this was a sum of the amplitudes of oscillations in each frequency bin, over a selected range of frequencies. The width of our frequency bin was 0.06 Hz, which was a function of the length of time data was sampled. The units of AUC are therefore mm × Hz/0.06. Because of the cumbersome nature of these types of units, however, pupillary unrest is customarily quantified in arbitrary units (AU), a convention that we followed.

Initially, the selected frequency range was the entire dynamic range of the device, from 0 to 16 Hz. However, as the study progressed we learned that the AUC above 2.7 Hz was not different from the AUC observed from the measurements of metal holes. In addition, there appeared to be frequent extraneous artifacts below 0.2 Hz that were unrelated to pupillary unrest. We therefore narrowed the relevant requency range for the AUC to 0.2–2.7 Hz.

Because blinks have been reported to alter the size of the pupil, we counted the number of blinks in our entire data sample of 103 measurements. The number of blinks in each measurement category (e.g. baseline 1, baseline 2, fentanyl, recovery, 25, 100, 250 and 500 lx) was also counted, as well as the direction of pupil movement after each blink.

2.4. Statistical analysis

For statistical analysis of pupil size and PUAL before and after fentanyl and at different light intensities we used repeated measures ANOVA with Tukey's correction for multiple comparisons. We also used ANOVA with Tukey's corrections to compare the values of the average of the two baseline measurements to those taken 4–5 min after fentanyl and to those during the recovery phase in those selected patients whose induction of general anesthesia was delayed. The number of blinks in each category of the drug study was compared using repeated measures ANOVA with Tukey's corrections. A similar analysis was performed for the separate study with varying light intensities. Pearson correlation coefficients were calculated to assess the relationship between the change in PUAL to that of the change in pupil size brought about by fentanyl.

3. Results

No patients refused to continue participation in the study after the first measurement was taken. The age of the patients in the completed fentanyl study was 40 ± 12 years and weight was 82 ± 10 kg (M = 14, F = 6). Volunteers who participated in the study that measured the effect of ambient light on PUAL and pupil size were 36 ± 14 yr old and weighed 79 ± 12 kg (F = 4, M = 6).

3.1. Calibration and effect of light

Repeated measurements of test metal holes yielded values to within 0.1 mm of the calibrated diameters. The sum of FFT amplitudes between 0.2 and 2.7 Hz for metal holes was consistently below 0.1 (units are arbitrary units as discussed in the Methods section). Movement of the metal holes during measurement added value to the area under the curve of the FFTs but there was accurate tracking of the target metal aperture, and AUC between 0.2 and 2.7 Hz with movement did not exceed 0.15 A.U.