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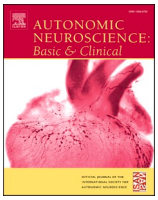

Detection of opioid effect with pupillometry☆

Rachel Eshima McKay, Merlín D. Larson *

Department of Anesthesia and Perioperative Care, University of California San Francisco (UCSF), San Francisco, CA, United States of America

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ABSTRACT

Background: Opioids produce pupillary constriction but their impact on pupillary unrest and the dynamic parameters of the pupillary light reflex have not been characterized. Given the increasing use of portable pupillometers for care of critically ill patients, it is important to distinguish between opioid effects on the pupil versus those that have been reported to arise from traumatic and ischemic brain insults. We undertook this study to determine which pupillary responses are most profoundly and consistently affected by a progressive infusion of remifentanil.

Methods: We studied the effect of remifentanil on the pupil using two portable infrared pupillometers in 18 volunteers. One pupillometer measured pupillary unrest in ambient light (PUAL) and the other pupillometer measured neurological pupillary index (NPi), constriction velocity (CV), pupil diameter (PD), latency, and % reflex (% reflex) following a transient light flash. Remifentanil was administered at predetermined weight-adjusted rates to raise opioid effect site concentration up to a range known to produce respiratory depression and oxyhemoglobin desaturation, based on a previously published pharmacokinetic model.

Results: PUAL was ablated by remifentanil, declining 94 ± 6% from baseline at the time of maximum drug effect. Other pupillary measurements decreased 50–65% from baseline. NPi was unchanged. At the time of oxyhemoglobin desaturation, deviations in PD, CV, and % reflex were widely scattered, whereas PUAL consistently approached zero.

Conclusion: PUAL is a highly specific indicator of central opioid effect. As a non-invasive measure, it may provide useful data to clinicians who prescribe opioids.

1. Introduction

There is a continuing need for clinical measures to assess opioid effect (Lee et al., 2015; Overdyk et al., 2018). Pupil diameter (PD) has been a previously cited measure of opioid effect, but miosis as a clinical sign is not specific for opioids (Boyer, 2012). Within the past ten years portable infrared pupillometers that can measure the pupil in darkness have become available (Larson and Behrends, 2015). These devices can quantify pupillary unrest and several parameters of the light reflex waveform. Dynamic measurements including pupillary unrest, reflex amplitude (RA), constriction velocity (CV), and percent light reflex (% reflex) (see Fig. 1 for explanation of terms) have been proposed as more precise measures of opioid effect compared to PD (Pickworth et al., 1989; Pickworth and Fudala, 1990; Bokoch et al., 2015; Kongsgaard and Hoiseth, 2019; McKay et al., 2018; Rollins et al., 2015).

Opioids have been shown to depress oscillatory motions of the pupil in awake subjects. These oscillatory motions are referred to as pupillary unrest in ambient light (PUAL) and are thought to arise from opposing inhibitory and excitatory influences on the Edinger-Westphal (EW) nucleus (Bokoch et al., 2015; Turnbull et al., 2017; Smith et al., 1970). In a previous case report we observed opioid induced ablation of PUAL concurrently with severe respiratory depression, which suggested to us that PUAL has an absolute lower-limiting boundary associated with opioid toxicity (McKay et al., 2018). We conducted the following study to determine which parameters measured from the pupil are most significantly altered by high concentrations of remifentanil and if any measures would predict profound toxic respiratory depression.

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* Corresponding author at: Department of Anesthesia and Perioperative Care, University of California, San Francisco, CA 94143-0648, United States of America.
E-mail address: merlin.larson@ucsf.edu (M.D. Larson).

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2. Materials and methods

2.1. Study subjects

After receiving approval from our institutional review board (Human Research Protection Program, University of California, San Francisco, California), we conducted a study in 18 healthy volunteers following the Code of Ethics of the World Medical Association (Declaration of Helsinki). Subjects were admitted to Department of Anesthesia Volunteer Study Laboratory after an 8-hour fasting period. Lighting conditions (200 lx) were strictly controlled, and the room was free of distracting noise. Two board-certified anesthesiologists, equipped with standard resuscitation medications and supplies attended at all times. We chose to study 18 volunteers, a higher number of subjects than were studied in previous investigations that demonstrated consistent pupillary responses to infusions of remifentanil (Barvais et al., 2003; Rollins et al., 2015).

2.2. Monitors

After providing consent, each subject received 40 mg aprepitant by mouth, a 20-gauge intravenous line in the hand or arm, 4 mg of intravenous ondansetron, and Ringer's Lactate solution by infusion at 150 cm^3/h. Monitors included non-invasive blood pressure, oxyhemoglobin saturation, electrocardiogram, end tidal carbon dioxide concentration, and transcutaneous CO2 (SenTec AG, Ringstrasse 39, CH-4106 Therwil Blvd, Switzerland).

2.3. Pupillary measurements

We used two different pupillometers. The Neuroptics PLR – 300 to measure PUAL and PD and the Neuroptics NPi- 200 (Neuroptics, Inc., 23041 Avenida de la Carlota, Laguna Hills, CA 92653) to measure latency, NPi, CV, and % reflex. The Neurological Pupillary Index (NPi) is a proprietary number that purports to gauge the quality of the pupillary light reflex that is independent of PD (Chen et al., 2011). All PUAL measurements were taken during photopic conditions similar to those encountered in a typical indoor environment. We did not perform dark adaptation, as we felt this procedure would be impractical for most clinical applications.

We used the Neuroptics PLR – 300 to measure PUAL. This instrument directs a soft halo of light through an occlusive rubber cup that was placed over the left eye. Illumination of the pupil is needed to initiate the characteristic fluctuations of PUAL (Warga et al., 2009) that are thought to arise from intermittent inhibition of the EW nucleus (Smith et al., 1970). Light directed into the measured left eye was provided by a soft blurred disk of white light from a 50 μ-watt source, at approximately 350-lux illumination. Previous studies have demonstrated that PUAL values decline progressively as light intensity exceeds 450 lx or falls below 100 lx (Usui and Stark, 1978; Bokoch et al., 2015; Behrends et al., 2018).

When using the Neuroptics PLR – 300 instrument on the left eye, the operator's left hand covered the subject's right eye to block out ambient light. The measurements took 10 s to complete and were processed post hoc to quantitate a PUAL measure, thereby blinding the investigators to those values during the study (Neice et al., 2017). Two baseline measurements of PUAL were taken from the left eye using methods of measurement that have been described previously (Neice et al., 2017). Calculation of PUAL was performed using the area under the curve of the Fourier transformation of pupil diameter fluctuations. The measurement, expressed in arbitrary units (AU), consists of amplitude summations between 0.3 and 3 Hz. (Neice et al., 2017; McKay et al., 2018).

We used a separate pupillometer (NPi–200) to evaluate several parameters of the pupillary light reflex waveform. This instrument is increasingly used in critical care settings, utilizing components of the pupillary light reflex, to detect and track severity of ischemic brain injury (McNett et al., 2018; Chen et al., 2011). In our study we took the light reflex measurements immediately after the PUAL measurement, and ambient light was again occluded from the right eye by the operator's left hand. The pupillometer was positioned over the left eye with a cheek rest, and after a three second pause delivered a light flash of 800-msec duration at 121 microwatts of energy. The light reflex settings on the NPi–200 are set by the manufacturer and cannot be altered. The pupillary light reflex waveform was measured for 3 s after the flash onset. The Neuroptics NPi-200 pupillometer automatically calculates baseline PD, NPi, CV, % reflex, and latency.

2.4. Drug infusion and recovery

Following these baseline pupillary measurements, a remifentanil infusion was started and maintained at 0.2 μg/kg/min for 5 min, then increased to 0.3 μg/kg/min for an additional 5 min, and then

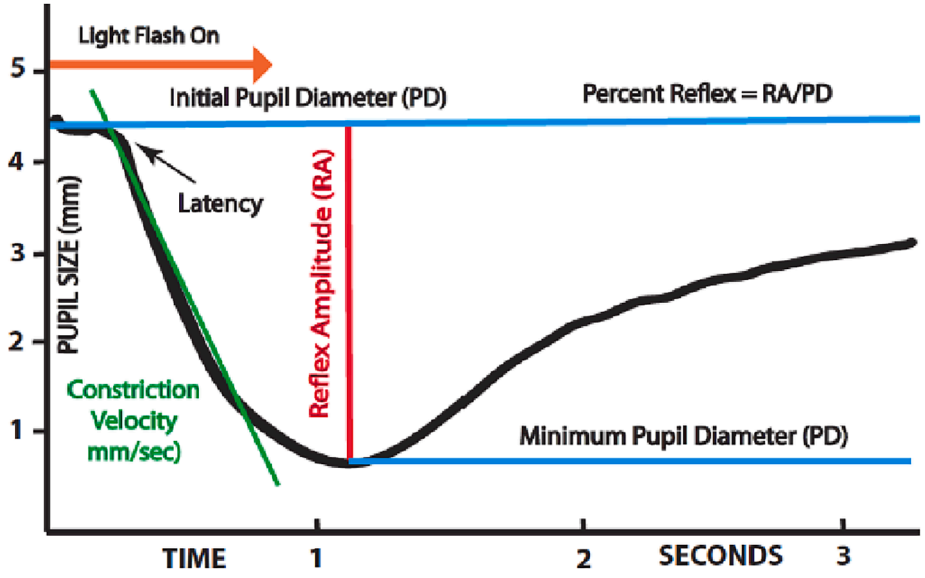

Fig. 1. Example of the pupillary light reflex waveform illustrating the parameters measured in this study. The Neuroptics portable infrared pupillometer samples at 30 Hz and has a precision of 0.03 mm. Percent reflex (% reflex) is reflex amplitude (RA) divided by initial diameter (PD).

discontinued. We selected an infusion scheme based on estimates from a previously published pharmacokinetic model (Minto et al., 1997) with the intention of meeting an effect site concentration of remifentanil between 4 and 6 ng/cm^3 at 10 min. Previous studies have reported that these concentrations would produce profound respiratory depression (Lang et al., 1996; Olofsen et al., 2010). After discontinuation of the remifentanil infusion, ongoing monitoring and data collection continued for 25 additional minutes.

PUAL and the parameters of the pupillary light reflex as described previously were taken at time 0, and every 2.5 min during the 10-minute infusion and 25-minute recovery period. Volunteers were engaged in conversation with the investigators, but not prompted to breathe at any time. We defined severe respiratory depression as rapidly declining oxyhemoglobin saturation below 90%, at which time supplemental oxygen at 2 l/min was initiated via nasal cannula. On occasions when SpO2 fell <90%, pupillary measurements were delayed for several seconds until oxyhemoglobin saturation rose to ≥90% by delivery of supplemental oxygen.

The investigators observed each volunteer for at least 1 h after the discontinuation of the remifentanil infusion. When volunteers were able to ambulate without dizziness and take clear liquids by mouth without prohibitive nausea, the intravenous catheter was removed and each subject was discharged into the care of a responsible adult.

2.5. Statistical analysis

We evaluated PUAL, PD, CV, NPi, latency, and % reflex taken every 2.5 min during the 10-minute infusion and the 25-minute recovery period. Differences between the baseline parameters and those obtained after 10 min of remifentanil infusion when opioid levels reached predicted maximum were evaluated with the Wilcoxon paired signed-rank test. The percent change in parameter at 10 min compared to baseline were calculated and compared using the Kruskal–Wallis test, after rejection of the Kruskal–Wallis null hypothesis. Pairwise comparisons between proportional change in each parameter were analyzed by the Dunn-test (Dunntest Package, Stata 16).

PUAL, CV, PD, NPi, latency, and % reflex at 10 min were compared between volunteers who desaturated and those who did not desaturate, using the Wilcoxon rank sum test.

To assess the predictive value of each pupillary parameter for the occurrence of desaturation, we calculated the area under Receiver Operating Characteristic (ROC) curves. For this analysis the state variable was the occurrence of desaturation and the dependent variables were the pupillary parameters. Results are expressed as area under the curve (AUC).

We constructed scatter plots for PD vs CV, PUAL vs PD, remifentanil effect site concentrations vs PUAL, PD vs % reflex, and RA vs CV. Quadratic, linear, and exponential regressions and trendlines were constructed. We performed data analysis using Stata Version 16 (College Station, TX).

3. Results

3.1. Demographics and vital signs

Demographics of the 18 volunteers were as follows: Age - 26 ± 4 yrs., Weight - 60 ± 11 kg, Body Mass Index - 22.3 ± 3.2 kg/m2, and Sex - 8/10, M/F. POSS scores ranged from 1 to 2 throughout the study. Although all volunteers remained in verbal contact with the investigators, there was significant respiratory depression observed in all cases. Average end tidal CO2 rose 8.9 ± 5.3 mm Hg, and respiratory rate decreased below 8 in 7 cases and below 4 in 2 cases. In 60% of the cases the respiratory pattern was irregular with brief episodes of rapid respiration followed by pauses of up to 15 s. Mean increases in end tidal CO2 values peaked at 15 min, 5 min after the remifentanil infusion was discontinued.

Nine of 18 volunteers required oxygen supplementation because of rapidly declining oxyhemoglobin saturation below 90%. These volunteers continued to respond to verbal commands and cooperate with the pupillary measurements. Satisfactory oxygenation was rapidly restored after the administration of nasal oxygen. Participants were discharged without incident on the same day and reported no complications when contacted by telephone the following day.

3.2. Pupillary changes after remifentanil

PUAL values before remifentanil infusion ranged between 0.12 and 0.45 AU. Remifentanil infusion resulted in significant decrease in PD, PUAL, CV, and % reflex (Table 1); NPi and latency did not change (Table 1 and Fig. 2). Percent decline was more pronounced for PUAL compared to PD or the excitatory reflexes (Table 1).

Differences at the 10-minute measurement in PUAL, PD, CV, NPi, and latency between those volunteers who exhibited oxyhemoglobin desaturation and those who did not desaturate are shown in Table 2. The difference in PUAL was significant. The ROC curve demonstrating the sensitivity and specificity for PUAL to predict oxyhemoglobin desaturation at the 10-minute interval is shown in Fig. 3. The performance of the PUAL diagnostic test for desaturation following a toxic remifentanil infusion is shown in this Figure by the Youden index. The AUC for PUAL at 0.94 was indicative of outstanding discrimination. AUC values for CV, PD, and % reflex were 0.78, 0.57 and 0.62 respectively.

The relationships between the various parameters, trendlines, and significance are shown in Table 3. Progressive increase in remifentanil led to an exponential decline in PUAL, to near total ablation. The best fits for RA vs CV, PD vs CV and PD vs % Reflex were quadratic (Table 3). The relationship between PUAL and PD was linear. An example of the decline in PUAL values in one volunteer is shown in Fig. 4.

The graphic analysis for CV and PD is shown in Fig. 5. The best-fitted curve represents a parabolic segment of a quadratic formula (Fig. 5B). CV values in the 9 volunteers who exhibited oxyhemoglobin desaturation on room air were all positioned within the range of other values from volunteers who did not desaturate. Fig. 5C and D illustrate the relationship between PD and % reflex. Consistent with CV findings during desaturation, % reflex was not ablated in the 9 volunteers whose saturations dropped rapidly below 90%. This relationship also shows a break from linearity below approximately 3 mm PD.

On the other hand, the relationship between PUAL and PD was linear, and PUAL values were essentially ablated when the opioid effect became toxic (Fig. 6). Fig. 7 shows all the individual values for PUAL and CV over the initial 12.5 min. At toxic doses of remifentanil, CV never approached zero regardless of the subject's baseline measurement (7A). However, volunteers who had high PUAL values before the drug were depressed to near zero in a similar manner to those whose baseline PUAL values were in the lower quartiles (Fig. 7B). One subject with a predrug pupil size of 2.9 mm had a PUAL value of 0.39 AU, and in this instance PUAL was depressed to zero AU after remifentanil.

4. Discussion and conclusion

We studied the pupillary effects of a progressively increasing concentration of remifentanil that produced respiratory depression and hypoxia in volunteer subjects. We demonstrated that at these toxic doses, opioid ablated PUAL but the excitatory reflexes persisted. We also demonstrated that the effects on latency and NPi were unchanged and that the depressant effects on CV, and % reflex were brought about by the decrease in PD.

There are conflicting messages in the literature regarding the effect of opioids on the pupillary light reflex (Kongsgaard and Hoiseth, 2019). Alfentanil given during general anesthesia did not alter the pupillary light reflex (Larson et al., 1997). Pickworth reported that opioids diminished both the PD and CV in awake subjects (Pickworth et al., 1989). Rollins demonstrated a 6% decrease in NPi and modest reductions in light reflex-related parameters in volunteers receiving

Table 1
Absolute and percent change in pupillary measurements at 10 min (peak estimated effect site concentration of remifentanil).

N = 18x	PLR-300 pupillometer		NPI-200 pupillometer			
Parameter Mean ± SD (Median) {25,75 %-ile}	PUAL (AU)	Diameter (mm)[#]	CV (mm/s)	% reflex	NPi®	Latency[$]
Baseline	0.276 ± 0.102 (0.28) {0.19, 0.33}	4.41 ± 0.56 (4.47) {4.24, 4.84}	3.30 ± 0.51 (3.44) {3.17, 3.84}	37.35 ± 4.81 (37) {36, 38}	4.18 ± 0.30 (4.2) {4.1, 4.3}	0.218 ± 0.018 (0.215) {0.20, 0.23}
10 min	0.016 ± 0.003 (0.02) {0.00, 0.02}	2.19 ± 0.26 (2.13) {2.05, 2.31}	1.12 ± 0.46 (1.15) {0.73, 1.48}	12.30 ± 5.03 (13) {7, 17}	4.24 ± 0.30 (4.2) {4.1, 4.5}	0.221 ± 0.022 (0.23) {0.20, 0.23}
Difference, Baseline vs 10 min[^]	P < 0.0001	P < 0.0001	P = 0.0020	P = 0.0020	P = 0.7266	P = 0.7266
10-Minute percent parameter decline:	93.96 ± 5.80[a] (94.27) {89.74, 100} Range: (83.33, 100) Rank Sum: 848.50	49.75 ± 6.91[b] (51.59) {47.62, 53.63} Range: (31.16, 59.78) Rank Sum: 234.00	65.24 ± 15.64[c] (67.57) {57.54, 76.94} Range: (34.93, 87.19) Rank Sum: 253.00	66.39 ± 15.72 (65.28) {52.78, 80.82} Range: (37.93, 85.07) Rank Sum: 260.50	NA	NA
Kruskal–Wallis Chi2 = 40.377, P = 0.0001 for decline between parameters at 10 min						

Post-hoc pairwise comparisons by Dunn-test.
a: PUAL decline at 10 min greater than
-Diameter decline (P < 0.0001)
-CV decline (P = 0.0020)
-% Reflex decline (P = 0.0031).
b: Diameter decline at 10 min did not differ significantly from
-CV decline (P = 0.1671)
-% Reflex decline (P = 0.1271).
c: CV decline at 10 min did not differ significantly from
-% Reflex decline (P = 1.0000).
d: PUAL decline at maximum change was greater than
-Diameter decline (P < 0.0001)
-CV decline (P = 0.0006)
-% Reflex decline (P = 0.0010).
e: Diameter decline at maximum change was less than
-CV decline (P = 0.0038).
Diameter decline at maximum change did not differ significantly from
-% Reflex decline (P = 0.0677).
f: CV decline at maximum change did not differ significantly from
-% Reflex decline (P = 1.0000)
[#] Mechanical limitations of the pupil prevent decrease to zero at maximum suppression.
[$] Delay of neuromuscular transmission prevents decrease to zero at maximum suppression.
[^] Wilcoxon paired signed-rank test.

prolonged remifentanil infusion with supplemental oxygen (Rollins et al., 2015). A recent review of pupillometry applications in the neurointensive care unit reported that opioids decrease the magnitude of the pupillary light reflex (Bower et al., 2019). With the increasing use of pupillary light reflex measurements in intensive care units (Du et al., 2005; Couret et al., 2016; Bower et al., 2019; Lussier et al., 2019), it is important to clarify just how opioids are affecting the pupil, and to distinguish opioid-related changes from early signs of increased intracranial pressure (McNett et al., 2018), or hemorrhagic/ischemic events in the midbrain (Bower et al., 2019; Lussier et al., 2019; Oddo et al., 2018; Shoyombo et al., 2018).

Because our cases were titrated into the range of dangerous opioid toxicity and we observed no change in NPi, we conclude that NPi changes cannot be attributed to opioid therapy. The light-stimulated measurements were never ablated, nor did they reach an unambiguous threshold in response to opioid infusion, even at doses that produced critical respiratory depression. This observation was not unexpected because others have shown that even with a less intense light flash, the pupillary reflexes are still intact during general anesthesia supplemented with opioids (Larson et al., 1997). Additional observations have shown that the pupillary light reflex is often intact during cardiopulmonary resuscitation (Behrends et al., 2015).

The effect of remifentanil on PUAL was such that near total ablation was achieved when oxyhemoglobin desaturation occurred during the remifentanil infusion. The significance of this finding becomes clearer when we consider that the smallest PD achievable for any individual is unknown; in our study pupil diameters at the time of oxyhemoglobin desaturation varied between 1.8 and 2.6 mm. The light-reflex related parameters were even less predictable, with CV ranging from 0.4–1.6 mm/s, and % reflex from 5 to 17%. In other words, the PD and excitatory reflexes are less valuable when one attempts to evaluate opioid toxicity because they continue to reliably report positive values even when opioids are titrated into dangerous concentrations. These dynamic light-induced values did not appear to be directly depressed by the drug, but instead in each individual subject they were affected only secondary to the decline in PD (Fig. 5). We contend that PD decreases because PUAL is progressively blocked and dangerous respiratory depression is likely if PUAL is totally ablated. Our idea is that when inhibition is totally blocked as observed by absent PUAL, the pupil does not constrict further unless activated by an increase in light intensity.

As pupil size decreases the RA diminishes because the small pupil has less range of motion compared to large pupils. We have confirmed previous reports that a non-linear relationship exist between RA and CV (Ellis, 1981; Bremner, 2012). This change in the relationship between

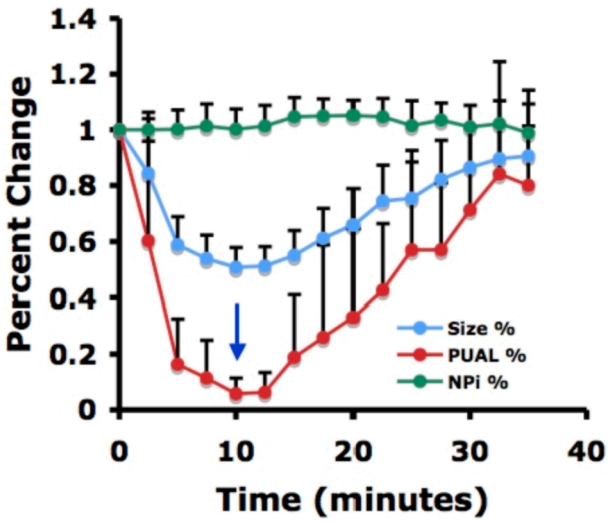

Fig. 2. Percent changes in PUAL, PD, and NPi after remifentanil infusion sufficient to produce significant respiratory depression. Blue vertical arrow represents the time when nine of 18 subjects exhibited rapid oxyhemoglobin desaturation. No change in NPi was observed. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Parameters at 10 min separated by those who remained saturated above 90% and those whose oxyhemoglobin saturation dropped below 90%.

N = 18x	PLR-300 pupillometer		NPI-200 pupillometer			
Parameter Mean ± SD (Median) {25,75 %-ile}	PUAL (AU)	Diameter (mm)[c]	CV (mm/s)	% reflex	NPi®	Latency[d]
10-Minute parameter:						
SpO2 < 90%, N = 9 (4.9)[a] {4.7–5.1}	0.009 ± 0.012 (0.00) {0.00, 0.02}	2.15 ± 0.16 (2.11) {2.17, 2.30}	0.88 ± 0.15 (0.89) {0.73, 1.14}	10.4 ± 4.8 (10) {7, 13}	4.25 ± 0.26 (4.3) {4.05, 4.45}	0.228 ± 0.02 (0.23) {0.23, 0.23}
SpO2 ≥ 90%, N = 9 (4.9)[a] {4.4–5.2}	0.022 ± 0.012 (0.02) {0.02, 0.02}	2.24 ± 0.33 (2.11) {2.05, 2.37}	1.36 ± 0.45 (1.48) {1.17, 1.71}	14.2 ± 5.0 (16) {13, 18}	4.24 ± 0.35 (4.1) {4.1, 4.6}	0.216 ± 0.025 (0.215) {0.23, 0.23}
Difference, ±Hypoxia[b]	P = 0.01	P = 0.66	P = 0.31	P = 0.22	P = 0.90	P = 0.76

[a] Estimated remifentanil effect site concentration (ng/cm^3).
[b] Wilcoxon rank sum test.
[c] Mechanical limitations of the pupil prevent decrease to zero at maximum suppression.
[d] Delay of neuromuscular transmission prevents decrease to zero at maximum suppression.

CV and PD, and between % reflex and PD at small diameters is thought to arise because of structural limitations brought about by the mechanical features of the iris (Chen and Kardon, 2013). On the other hand, PUAL is generated by simultaneous inhibition and excitation (Bokoch et al., 2015) and thus might entail less restriction of movement at small diameters. If either excitation or inhibition is decreased, then PUAL values diminish and are eliminated if either source is absent (Fig. 8).

An objective tool to measure opioid effect would be a useful addition to the management of opioid medicated patients. Many of the clinical signs of opioid toxicity can be obscured by environmental stimulation and use of supplemental oxygen, or can be confused with neurologic decompensation related to brain ischemia. Miosis by itself has proven to be an unreliable clinical measure of opioid effect because it is affected by

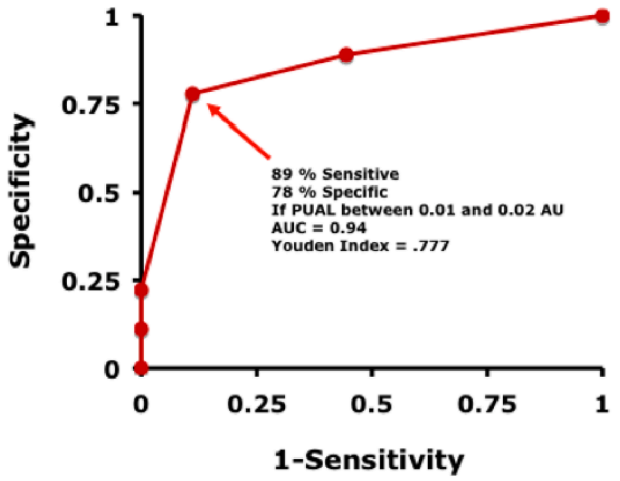

Fig. 3. PUAL as a predictor of severe opioid toxicity. Shown is the ROC curve with AUC at 94 and the Youden Index with 89% sensitivity and 78% specificity.

Table 3
Regression curves for pupillary data after remifentanil.

Independent (x)	Dependent (y)	Formula	R^2	P value
Remifentanil effect site concentration (ng/cm^3)	PUAL	$y = 0.24e^{-0.38(x)}$	0.55	0.001
PUAL	PD	$y = 9x + 2.13$	0.99	0.0001
PD	% REFLEX	$y = -2.2x^2 + 24.6x - 31.6$	0.96	0.00001
RA	CV	$y = -.54x^2 + 2.7x + 0.32$	0.90	0.00001
PD	CV	$y = -0.18x^2 + 2.2x - 2.99$	0.98	0.00001

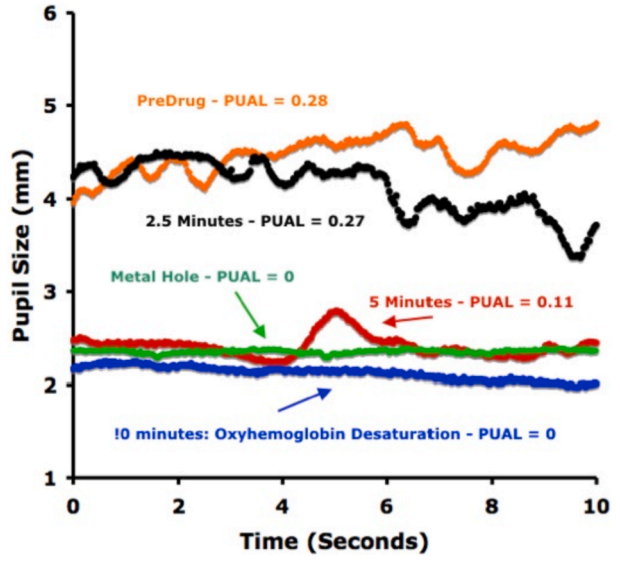

Fig. 4. A typical case showing the changes in PUAL after remifentanil infusion. Nasal oxygen was added at 10 min because of oxyhemoglobin desaturation in rapid decline below 90%. Note the small fluctuations in PD during the 10 min measurement that represents noise in the measuring device. A measurement from a metal hole of similar diameter is shown for comparison.

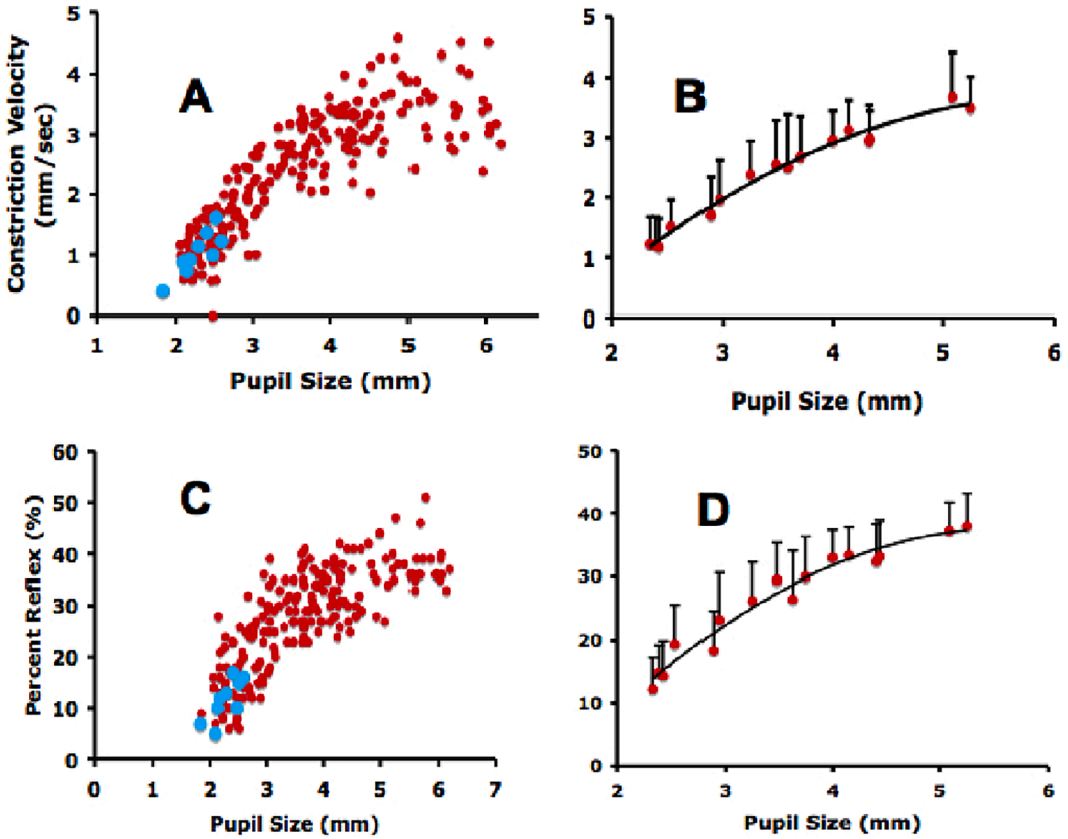

Fig. 5. A: Scatter plot showing the relationship between CV and PD for all measurements. PD is the independent variable. The blue markers designate the values of those volunteers that exhibited oxyhemoglobin desaturation. B: Each infusion of the drug had 15 measurements. This graph shows the relationship between PD and CV at each 2.5 min interval between 0 and 35 min. Each data point is the mean of all 18 volunteers. The best fit of the data is a quadratic equation with the slope increasing at small pupil diameters. The correlation coefficient is highly significant ($P < 0.001$).
C. Scatter plot showing the relationship between % reflex and PD for all measurements. The blue markers indicate the values of those volunteers that exhibited oxyhemoglobin desaturation. D. Relationship between PD and % reflex at each 2.5 min interval between 0 and 35 min. PD was the size in mm during the latency period. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

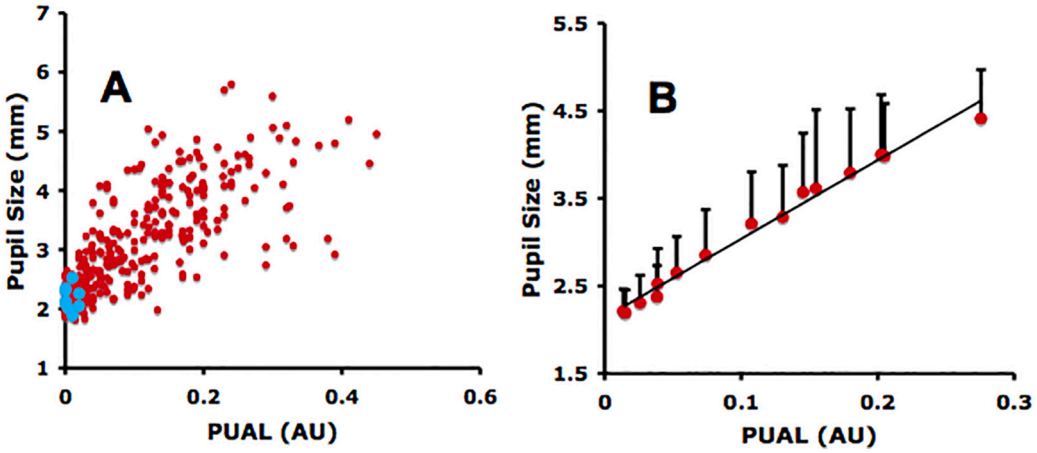

Fig. 6. A: Scatter plot relating PUAL and PD for all subjects. PUAL is the independent variable. PUAL is essentially blocked by remifentanil when the drug reached toxic levels (oxyhemoglobin desaturation) as shown by the blue markers. B: Each infusion of the drug had 15 measurements. This graph shows the relationship between PUAL and Pupil Size at each 2.5 min interval between 0 and 35 min. Each data point is the mean of all 18 volunteers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

light, near fixation, other comorbid conditions, and lack of a precise lower-limiting boundary (Boyer, 2012). For this reason we suggest PUAL is a more valid measure of opioid effect than either PD or the excitatory reflexes.

A limitation to this study is that we measured the pupil in volunteers who were pain free, without co-morbidities, and not taking other medications. Mild sedation with midazolam has been shown to have no appreciable effect on PUAL (Behrends et al., 2018) but propofol in doses that produce unresponsiveness will depress PUAL (Behrends et al., 2018). PUAL represents the movements of smooth muscles activated by transmission through the 3rd cranial nerve. Therefore conditions that interfere with 3rd nerve function such as uncal herniation, Adie's syndrome, advanced diabetic neuropathy and topical anticholinergics might also depress PUAL.

In clinical practice we have observed subjects who are in severe pain but still have absent PUAL values after opioid therapy (McKay et al.,

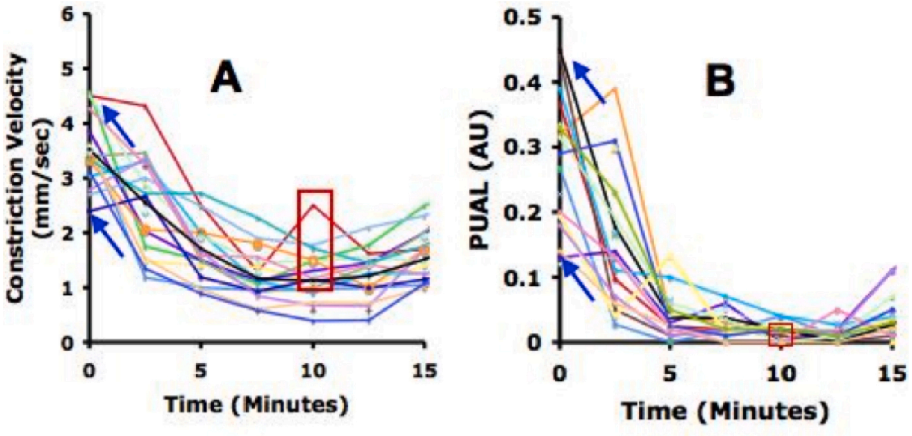

Fig. 7. A: Measurements of CV during remifentanil infusion. Large and small (blue arrows) initial values of CV remained positive (red rectangle) as drug levels became toxic. B: Measuremets of PUAL during remifentanil infusion. Large and small (blue arrows) initial values of PUAL were nearly ablated (red rectangle) as the drug became toxic. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

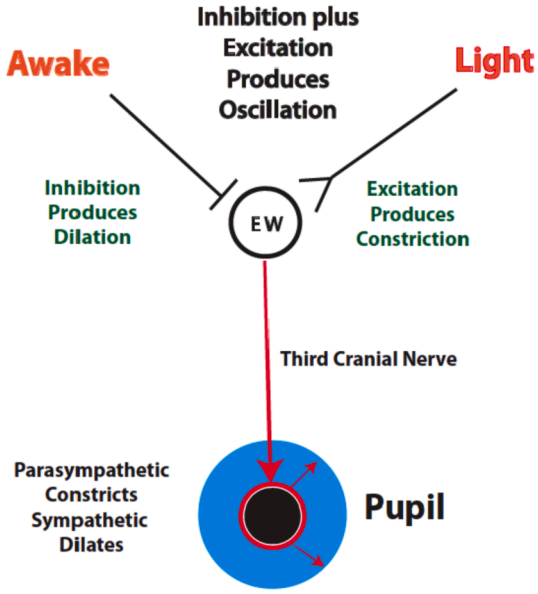

Fig. 8. The pupil oscillates when there are simultaneous excitatory and inhibitory afferents into the EW nucleus. Opioids block the inhibitor input and abolish PUAL. Light "off" abolishes excitation and blocks PUAL Without inhibitory or excitatory input, the pupil is miotic because of the intrinsic pacemaker activity of the E.W. nucleus (Ichinohe and Shoumura (2001)).

2018). Our contention is that opioids block the inhibitory influences on the EW nucleus that might arise from severe pain. The opposing influences of pain and opioid therapy (Charier et al., 2019) on PUAL are questions that deserve additional study.

The exact location of the inhibitory neurons responsible for the PUAL is unknown. We have previously observed and reported augmented values of PUAL in opioid tolerant patients as a component of the withdrawal syndrome (McKay et al., 2018). This finding could be consistent with increased locus coeruleus activity during cessation of opioids in chronic pain patients. The withdrawal syndrome is associated with heightened sympathetic activity (Akaoka and Aston-Jones, 1991; Kienbaum et al., 2002). While increased tension in the sympathetic dilator muscle of the iris might enhance the dilations brought about sphincter relaxation, (Bokoch et al., 2015; Loewenfeld, 1999; Chen and Kardon, 2013) consensus among investigators is that the origin of PUAL originates from oscillating activity within the EW nucleus itself, and not from phasic activity in the sympathetic innervation of the iris (Loewenfeld, 1999; Smith et al., 1968; Stark, 1969; Turnbull et al., 2017).

Oscillations In the central nervous system require a source of both inhibition and excitation (Buzsaki, 2006). When inhibitions at the EW nucleus are blocked by opioids (Larson, 2008), PUAL values diminish and are eventually ablated. Why the onset of severe opioid induced respiratory depression coincides with the ablation of PUAL is not known. One idea is that there are parallel pathways that produce PUAL and ventilatory effort, and opioids block both pathways. The locus coeruleus does have influences on PD (Aston-Jones and Cohen, 2005; Joshi et al., 2016) and respiratory drive, (Quintero et al., 2017; Meinychuk and Dockree, 2018; Pineda and Ahhajanian, 1997) but the exact role of this nucleus in interpreting our results cannot be addressed by our study.

In summary we have demonstrated the depression of PUAL to extinction is a consistent informative measure of opioid toxicity. On the other hand, the light induced reflexes of the pupil such as CV and % reflex are reduced only in proportion to the opioid induced change in PD.

Summary statement

We present evidence to support the value of pupillary unrest in ambient light as an assessment of central opioid effect.

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