



Suppression of pupillary unrest by general anesthesia and propofol sedation

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Abstract

The pupil undergoes irregular oscillations when exposed to light. These oscillations, known as pupillary unrest in ambient light, originate from oscillatory activity within the Edinger–Westphal nucleus in the midbrain. The midbrain and upper pons also contain nuclei known to be very sensitive to the effects of anesthetics that play a central role in maintaining wakefulness. We hypothesized that anesthetics may display similar effects on wakefulness and pupillary unrest. Repeat measurements of pupillary unrest using infrared pupillometry were performed in 16 patients undergoing general anesthesia and 8 patients undergoing propofol sedation. Pupil scans were analyzed using fast Fourier transformation to quantify the effects of the anesthetics on pupillary unrest. During general anesthesia and deep sedation, observed pupillary unrest values below 0.1 (AU) indicate complete suppression of pupillary oscillations. Pupillary unrest decreased more during general anesthesia [to 24% of baseline (95% CI 17–30%)] than pupil size [51% of baseline (95% CI 45–57%)]. Sedation with propofol was associated with a reduction in pupillary unrest that was correlated to the depth of sedation as assessed by the Richmond Agitation–Sedation Scale and the processed electroencephalogram. Pupillary unrest is caused by oscillatory activity within the midbrain that is affected by the state of wakefulness or by hypnotics directly. Increased sedation and general anesthesia reduce and then abolish pupillary unrest as wakefulness decreases. We speculate that midbrain nuclei responsible for wakefulness and pupillary unrest are either communicating or share a similar sensitivity to the effects of commonly used anesthetics.

Keywords Pupillary · Eye · Anesthesia · Propofol

1 Introduction

Pupillary unrest in ambient light (PUAL), also often called hippus, is defined as light-induced irregular fluctuations in pupil size, which appear to originate in the preganglionic portion of the Edinger–Westphal (pgEW) nucleus [1–4]. The opioid fentanyl depresses PUAL in human subjects [5], an effect thought to be mediated by the known action of opioids to depress inhibition into the pgEW nucleus [6]. Because

inhibition of the pgEW nucleus is strongly influenced by the state of wakefulness, and pgEW neuronal activity is very likely associated with the firing pattern of arousal centers in the brainstem, we hypothesized that PUAL would also be depressed during general anesthesia and sedation, and would provide a guide to the state of wakefulness. Specifically we asked whether PUAL was decreased during anesthesia and light sedation, whether the power frequency relationship was changed in a manner similar to the processed electroencephalogram, whether the changes in PUAL correlated with other measures of wakefulness, and whether any changes we observed were related specifically to an alteration in pupil size.

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2 Methods

2.1 Study subjects

After institutional approval and informed consent, we studied 16 patients undergoing arthroscopic knee procedures with general anesthesia and 8 patients undergoing total knee replacement with spinal anesthesia and propofol sedation. We excluded patients taking medications known to alter pupil size or pupillary reflexes, and patients with known eye diseases.

2.2 Study procedures

2.2.1 General anesthesia study

Patients were premedicated with 2 mg IV midazolam. General anesthesia was induced with intravenous fentanyl (1 mcg/kg body weight) and propofol (2–2.5 mg/kg body weight). After placement of a laryngeal mask airway, general anesthesia was maintained by sevoflurane administration. Intradermal lidocaine (1%) injections were made by the surgeon prior to all skin incisions. Additional fentanyl was administered as needed to control responses to surgical stimulation. Measurements of pupillary unrest in ambient light (PUAL) were made at baseline, following premedication, shortly after induction with propofol, prior to surgical incision, after the patients opened their eyes spontaneously in the recovery room, and before discharge. Pupillary measurements were only performed when the patient's cooperation or an adequate level of anesthesia was established that would allow retraction of both eyelids without squinting or movement of the head.

2.2.2 Propofol sedation study

After baseline pupillary measurements, 1–2 mg IV midazolam was administered for anxiolysis, followed by spinal anesthesia with 15 mg hyperbaric bupivacaine. In addition to standard ASA monitors, bispectral index (BIS) EEG (Medtronic, Minneapolis, Minnesota, USA) electrodes were placed [7]. A propofol infusion was started at a rate of 25–100 mcg/kg/min; no opioids were administered. The processed EEG was continuously recorded and intermittent measurements (every 10–15 min) of PUAL were taken during the surgical procedure. Depth of sedation was independently assessed by the attending anesthesiologist using the Richmond Agitation–Sedation Scale (RASS) [8]. Measurements of PUAL were blinded until the Fourier transforms were completed post hoc in both arms of the study.

2.3 Infrared pupillometry

Measurement of PUAL was performed with a modified commercial portable infrared pupillometer (Model RD-NL013, Neuroptics, Irvine, California) [5]. A constant source of diffuse blue light with an intensity of 100 lx and wavelength of 460 nanometers, covering 20° of the visual field, was directed into the left pupil. The light source was 2.5 cm from the corneal surface and this light was directed into the open left non-measured eye. Ambient light was excluded from the right eye with a rubber cup projecting from the lens of the pupillometer. An image of the pupillometer in use has been published [9]. 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 samples per second (0.03 s between sample times). Video recordings of PUAL can be viewed in the electronic version of this manuscript (see supplemental data). The average pupil diameter over the 16-s measurement was calculated and reported as pupil size for that measurement. During measurements of unresponsive patients, both upper eyelids were retracted so that light could enter the non-measured eye.

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, Merritt [10, 11] and Ludtke [12] to remove artifacts that did not arise from actual movements of the pupil, as well as slow drifts in diameter that do not constitute pupillary unrest. Drift in pupil size was reduced by elimination of low frequency components of the scan with a Gaussian filter, and blinks or partial closures were eliminated automatically by identifying discontinuities in the pupil scan. Following this, we used fast Fourier transformation (FFT) 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 [13]. Our measure of PUAL was the sum of amplitudes across specific frequencies (vide infra).

In order to establish noise and baseline stability of the measurements done with the portable infrared pupillometer, preliminary measurements had been performed on metal holes of 2.6, 3.6 and 4.8 mm diameter. The metal hole was moved during these recording to assess the ability of the pupillometer to track the aperture and provide reliable readings during spontaneous eye movements (see supplemental videos).

PUAL was quantified by calculating the area under the curves (AUC) of the FFTs 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 that 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 reported as arbitrary units (AU), a convention that we followed. We detected frequent extraneous artifacts below 0.3 Hz that were unrelated to pupillary unrest and we had established in an earlier study that the AUC above 3 Hz was not different from noise found in our metal holes measurements [5]. We therefore narrowed the frequency range used for analysis of the AUC to 0.3–3 Hz, a narrower range of frequencies than in our previous study [5].

The pupillary light reflex can confound PUAL measurements because pupillary unrest is defined as a chaotic variation not linked to any acute stimulus such as variation in light level, or extraneous artifacts cause by head movements. We repeated all measurements that were affected by extraneous stimuli, measurements with blinks that exceeded one every 5 s, and those with prolonged eyelid closures or head movement.

2.4 Statistical analysis

Prior to initiation of the study we determined that the effect size of our observations had to be large with high reproducibility to make it a useful observation with high predictive value. If the effects of sedation and general anesthesia could not be observed in a small number of patients, the predictive value of the measurements would be unacceptable for a potential clinical use.

In the general anesthesia study we compared PUAL and pupil size at defined time points of the case as outlined earlier, using a one-way analysis of variance (ANOVA) for repeated measures. A Bonferroni post hoc test was used for pair wise comparisons.

In the sedation study we used a general linear mixed model to analyze the correlation of BIS, PUAL, and pupil size with RASS to account for both the effects of the fixed factor RASS and the random factor of different individual subjects. Pairwise comparison was performed using ANOVA allowing for within person correlation.

The program used for statistical analysis was Stata (Version 14.1, StataCorp, College Station, TX).

3 Results

Patient demographics in our two patient studies differed as expected based on the procedures performed. Patients scheduled for total knee arthroplasty that enrolled in the sedation study were older (58 ± 13 vs. 40 ± 12 years old), and had higher ASA scores (range II–III vs. I–II) than those patients with arthroscopic knee surgery that enrolled in the general anesthesia study. 4 out of 8 patients in the sedation study were women, versus 5 out of 16 in the general anesthesia study.

3.1 General anesthesia study

Single measurements were taken at each interval and no measurements were discarded or repeated. Premedication with 2 mg IV midazolam moderately decreased PUAL but this change was not statistically significant. This midazolam dose also did not constrict the pupils (Table 1). Induction of general anesthesia with propofol resulted in an immediate suppression of PUAL, at all frequencies. Maintenance of general anesthesia with the volatile anesthetic sevoflurane resulted in an ongoing suppression of PUAL. Sevoflurane concentrations were $0.1 \pm 0.3\%$ (0.1 ± 0.2 MAC adjusted for age) after LMA placement and $1.4 \pm 0.3\%$ (0.8 ± 0.1 MAC adjusted for age) prior to skin incision. Pre-incision

Table 1 PUAL and pupil size changes in the general anesthesia study

N = 16	Baseline before premedication	After midazolam premedication	After induction of GA	Before incision	After Awakening in PACU	Before Discharge
PUAL (AU)	0.307 (0.251–0.363)	0.259 (0.220–0.297)	0.087 (0.069–0.105)**.##	0.061 (0.053–0.069)**.##	0.186 (0.135–0.238)**.&.&.\$	0.166 (0.134–0.198)**.#.&.\$
Pupil size (mm)	4.06 (3.63–4.50)	4.08 (3.70–4.45)	2.51 (2.25–2.77)**.##	2.00 (1.79–2.28)**.##	3.10 (2.80–3.40)**.#.\$	3.04 (2.78–3.30)**.##.\$

PUAL and Pupil size values at various stages of the anesthetic. Mean value and 95% confidence intervals are shown. Symbols indicate significant differences between measurements using a one-way ANOVA for repeated measurements with Bonferroni correction

**p < 0.005 versus baseline

p < 0.05 versus premedication

p < 0.005 versus premedication

& p < 0.05 versus after induction

\$ p < 0.05 versus before incision

\$\$ p < 0.005 versus before incision

measurements were indistinguishable from our preliminary measurements that had been performed on metal holes of various diameters. Pupil size also decreased during general anesthesia, albeit to a lesser degree. PUAL returned after emergence from general anesthesia without reaching baseline values, probably as a consequence of perioperatively administered opioids and residual sedation following general anesthesia. PUAL and pupil size changes over the course of the anesthetic are shown in Table 1. A representative case is illustrated in Fig. 1 and selected population FFTs are shown in Fig. 2. Pupillary reflex dilation that followed a painful intraoperative stimulus did not exhibit increased PUAL values compared to the preincision values for the same

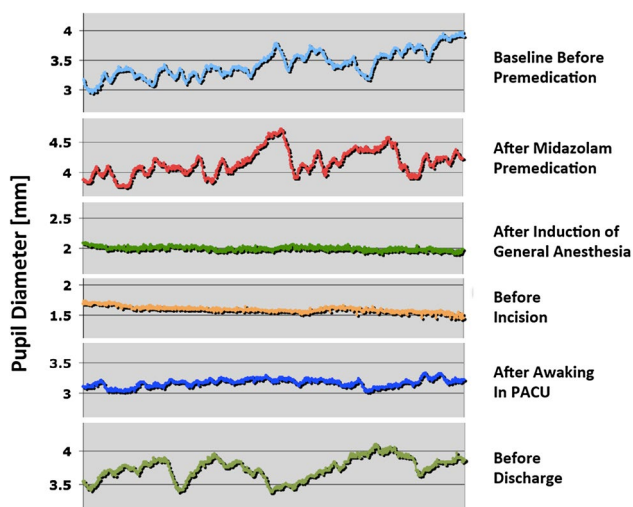


Fig. 1 Representative scans in one patient that demonstrates the changes in PUAL and pupil size at different stages of the general anesthesia study. The scans were taken before premedication, after administration of 2 mg IV midazolam, moments after induction of general anesthesia with fentanyl and propofol and the insertion of the laryngeal mask airway (LMA), before skin incision, in the recovery room after the patient opened his eyes for the first time spontaneously and before discharge from the ambulatory surgery center

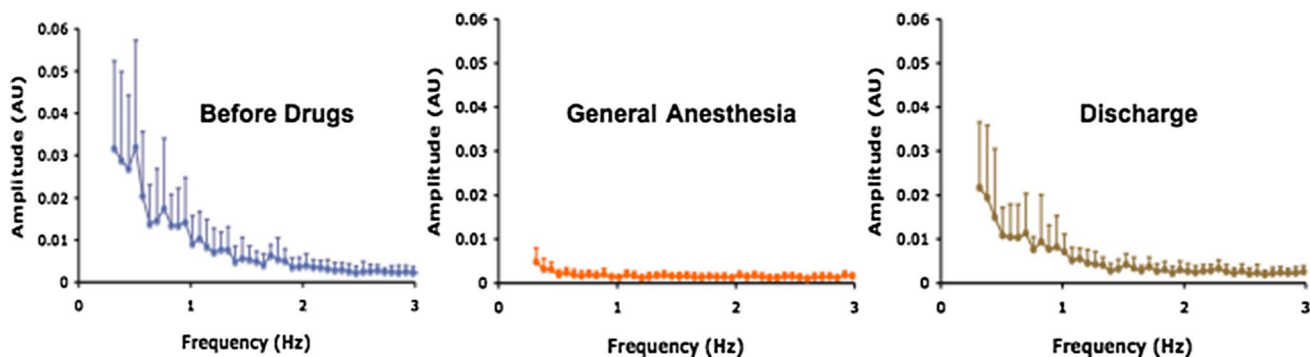


Fig. 2 Changes in fast Fourier transforms of pupillary unrest before, during, and after general anesthesia. Each line represents the average with error bars indicating the standard deviation in 16 study patients

patient (Fig. 3), demonstrating the ability of our algorithm to eliminate low frequency dilations (0.0–0.3 Hz) caused by surgical noxious stimulation that were unrelated to PUAL (0.3–3 Hz).

3.2 Sedation study

We took 128 measurements in 8 patients during various stages of sedation. Ten of these measurements had to be discarded because of excessive head motions, prolonged eye closures, or light induced changes that resulted in wide diameter swings unrelated to unrest. We did not reject measurements with pupillary reflex dilation because our applied filter was able to eliminate spurious values related to gradual dilations or constrictions (Fig. 3).

Propofol sedation during spinal anesthesia produced a gradual loss of PUAL as RASS scores decreased, as shown in Table 2 and Fig. 4. The correlation was most pronounced between suppression of PUAL and the decrease in sedation score ($p < 0.001$) (Fig. 4), but also highly significant for BIS vs. RASS ($p < 0.001$), pupil size versus RASS, ($p < 0.001$), as well as BIS versus PUAL ($p < 0.001$). Comparing PUAL at different levels of sedation demonstrates a profound drop in PUAL starting at a RASS of -2 when patients are no longer awake. Increased levels of sedation lowered PUAL further.

4 Discussion

The present investigation confirmed our primary hypothesis that PUAL would be depressed by general anesthesia. Within seconds of inducing general anesthesia a complete cessation of pupillary oscillations was seen. Furthermore, the findings of the sedation study suggest that the suppression of pupillary oscillations is correlated to the decrease in wakefulness of the patient. The reduction in pupillary oscillations with increased levels of sedation was seen reproducibly in

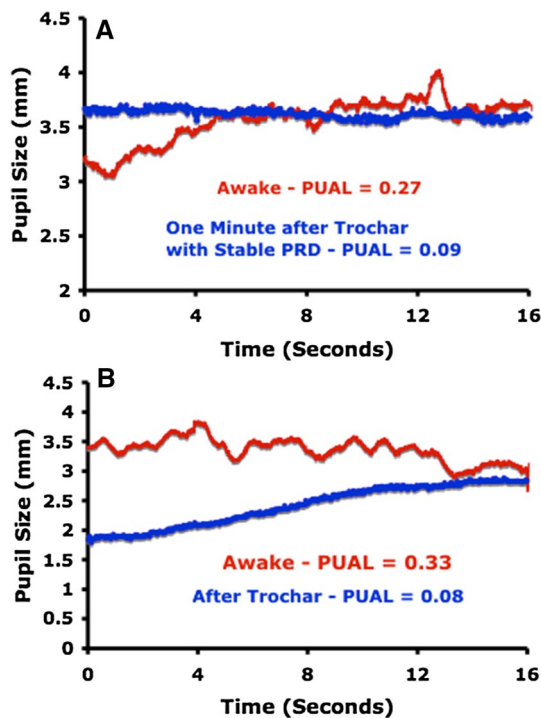


Fig. 3 Representative scans in patients with pupillary reflex dilations during the general anesthesia study. **a** During surgery under general anesthesia, a painful stimulus (trocar insertion) resulted in persisting pupil dilation to a diameter equal to the awake diameter. PUAL remained depressed indicating that PUAL suppression is not simply secondary to constriction of the pupil seen during general anesthesia. **b** After a painful stimulus (trocar insertion) there was a brisk dilation without oscillations. The PUAL measured at this event was equal to the preincision value, indicating that our algorithm is able to quantify PUAL while the pupil dilates to a noxious stimulus

all patients investigated with a sharp decline of oscillation as the patients fell asleep.

This reduction does not depend on a small pupil size because dilation of the pupil by noxious stimulation does not increase PUAL. These observations reinforce the concept that neuronal centers associated with awareness spontaneously inhibit the EW nucleus and are progressively depressed by sedative agents such as propofol.

The evidence that PUAL is mediated through oscillating activity within the EW nucleus is substantial. It is blocked by topical tropicamide, an agent that interferes with acetylcholine transmission at the pupillary sphincter [14]. Agents that are known to block inhibition at the pgEW nucleus [6] also reduce PUAL in awake subjects [5].

Our results contrast sharply with effects of drowsiness on pupillary unrest when measured in total darkness. The pupil in darkness exhibits only very small amplitude oscillations (video 1 in supplementary material) and therefore pupillary unrest is essentially absent [15]. An alert individual is able to maintain a large pupil in darkness until drowsiness ensues, at which time transient micro-arousals interrupt the trend toward the complete miosis of sleep. A fluctuating pupil size is therefore produced and has been measured with an algorithm called the pupillary unrest index [16].

Pupillary unrest in ambient light that we have measured is increased by near fixation and by increased light intensity, both of which increase parasympathetic tone [2, 17]. General anesthesia and propofol sedation also increase parasympathetic tone but *decrease* pupillary unrest. This contradiction can be explained by the differing mechanisms that influence PUAL in each condition. Propofol sedation and opioids

Table 2 PUAL and pupil size and BIS changes in the sedation study

8 patients 118 observations	RASS: 0 N = 11	RASS: - 1 N = 16	RASS: - 2 N = 7	RASS: - 3 N = 13	RASS: - 4 N = 29	RASS: - 5 N = 42
PUAL (AU)	0.252 (0.235–0.285)	0.260 (0.236–0.288)	0.148 (0.106–0.178)**##	0.136 (0.105–0.162)**##	0.087 (0.067–0.112)**##,%,%	0.093 (0.064–0.105)**##,%,%
Pupil size (mm)	3.64 (2.93–3.98)	3.10 (2.64–3.63)	2.77 (1.98–3.14)**##	2.86 (2.26–3.29)**	2.06 (1.75–2.67)**##,%,%	2.20 (1.56–2.47)**##,%,%
BIS	93.0 (86.7–101.6)	85.8 (77.8–90.8)*	80.3 (70.1–88.3)*	80.2 (74.2–88.4)*	67.3 (63.7–74.7)**##,%,%	60.7 (56.1–66.0)**##,%,%,&

PUAL, pupil size and BIS values at various depths of sedation. Mean values and 95% CI are shown. Symbols indicate significant differences in measurement results between sedation scores using the RASS score. A general mixed linear model was used, followed by pairwise comparison using ANOVA allowing for within person correlation

*p < 0.05 versus RASS: 0
 **p < 0.005 versus RASS: 0
 # p < 0.05 versus RASS: - 1
 ## p < 0.005 versus RASS: - 1
 § p < 0.05 versus RASS: - 2
 §§ p < 0.005 versus RASS: - 2
 % p < 0.005 versus RASS: - 3
 & p < 0.05 versus RASS: - 4

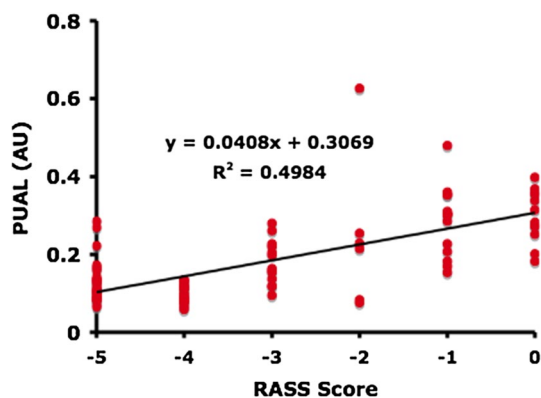


Fig. 4 Scatter plots demonstrating the relationship of PUAL to RASS scores during propofol sedation. Displayed are the results of 118 measurements in 8 patients

[5] block the inhibitory source of PUAL whereas increased parasympathetic tone by light and by near fixation both increase the excitation of the EW nucleus. Neuronal oscillations require both an excitatory and an inhibitory input [18].

The exact source of inhibition into the pgEW nucleus is unknown. Activation of hypothalamic and neocortical centers can induce pupillary dilation. These influences are presumably mediated through the upper pons because transection of the midbrain below the nucleus of the third cranial nerve results in temporary miosis and the behavioral signs of deep barbiturate anesthesia [19, 20]. Smith and Stark have described a group of neurons within the periaqueductal gray matter that inhibit the pgEW nucleus [21]. In addition, the locus coeruleus is thought to indirectly inhibit the pgEW nucleus [22, 23].

We have yet to explain why the inhibition exerted on this nucleus by a noxious stimulus during anesthesia pupillary dilations are unable to make the pgEW nucleus oscillate even in the presence of light. The nociceptive spinotectal pathway that generates pupillary reflex dilation is likely to be distinct from the neuronal centers that maintain the awake state, but we can only speculate why these two inhibitory pathways have differing effects on pgEW oscillations. It is significant that pupillary dilation following noxious stimulation is not able to trigger oscillations during anesthesia, whereas pupillary dilation from painful stimuli in the awake state has been reported to increase the amplitude of pupillary unrest [24].

The present study is the first to establish the link between changes in pupillary oscillations in light and wakefulness. We observed a gradual decrease in PUAL with increasing sedation produced by propofol infusions and a complete cessation of pupillary oscillations during general anesthesia. These findings suggest a connection between the regulation of wakefulness and the source of the irregular oscillation that manifest as PUAL. A further refinement of this

relationship might be obtained by comparing PUAL values to effect site propofol concentrations [25], but we elected to use clinical measures of sedation instead.

PUAL originates in the midbrain at a site from where it is difficult to detect steady state neuronal activity without the use of invasive electrodes. The EEG measures primarily cortical function and is only weakly affected by nociception and opioid effects that are mediated by brain stem structures. PET scans and functional MRI are potential tools to monitor midbrain activity but are cumbersome and costly. Observation of pupillary behavior may thus provide an additional clinical tool for monitoring midbrain function.

Pupillometry has applications in the operating room, the intensive care unit [26], the post anesthesia recovery room, and on acute pain rounds [9]. PUAL measurements are especially useful on the acute pain service and in paralyzed or locked-in patients as a measures of sedation and opioid effect [27]. The unique value of PUAL is in conjunction with other pupillographic measures that have been previously described [9]. PUAL considered together with the pupillary light reflex and pupillary reflex dilation allows the observer to assess the relative contributions of hypnosis and opioid effect. Volatile agents and propofol depress PUAL and the pupillary light reflex [28] but leave pupillary reflex dilation intact [29], whereas opioids have no effect on the pupillary light reflex [30] but depress both pupillary reflex dilation [31] and PUAL [5]. Infrared pupillometry has the potential to become a useful tool that could supplement established measures of wakefulness, opioid effect, and depth of sedation.

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Compliance with ethical standards

Conflict of interest Author Neice holds a patent related to signal processing of pupillary oscillations. No other authors have conflicts of interest.

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