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# The effect of ketamine and nitrous oxide on the human pupillary light reflex during general anesthesia ☆

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ABSTRACT

The neurotransmitters and receptor types involved in the afferent arm of the human pupillary light reflex are unknown. We hypothesized that the pupillary light reflex is mediated in part by NMDA receptors and that it would be depressed by the NMDA antagonists, nitrous oxide and ketamine. To study this question, sixteen patients received general anesthesia with desflurane, fentanyl, and muscular relaxation with rocuronium. After a stable level of general anesthesia had been obtained and at least 1 h after the start of the surgical procedure, ketamine 1 mg/kg ($N=8$) or saline ($N=8$) was injected intravenously by random selection. Heart rate, pupil size, pupillary light reflex, BIS scores, and blood pressure were measured every 2 min before and for 30 min after drug administration. A similar study of sixteen patients was then conducted with either addition of 60% nitrous oxide or 60% nitrogen to the gas mixture. We observed that the pupillary light reflex was depressed by ketamine and nitrous oxide by approximately 50%. The BIS score, representing the processed electroencephalogram, was elevated by ketamine and unchanged with nitrous oxide. Heart rate, pupil size, and blood pressure were unchanged by the drugs when compared to the control groups. We conclude that the two NMDA antagonists ketamine and nitrous oxide depress the human pupillary light reflex during general anesthesia whereas other monitored parameters were either unchanged or paradoxically elevated by the drugs. These findings present evidence that glutamate NMDA receptor activation is involved in generating the human pupillary light reflex.



## 1. Introduction

The neurotransmitters and receptors that elicit the human pupillary light reflex are unknown. Although two cholinergic synapses are present along the efferent pathway, the transmitters and receptor types along the afferent portion of the reflex have not been identified. Glutamate transmission has been demonstrated in the mammalian retina (Peng et al., 1995; Matsui et al., 1998; Kalbaugh et al., 2009) and kainate, alpha-amino-3-hydroxy-methyl-4 isoxazole proprionic acid (AMPA) and N-methyl-D-aspartic acid (NMDA) receptors have been demonstrated in the rodent suprachiasmic nucleus (SCN) (Pennartz et al., 2001). Because the SCN and the olivary pretectal nucleus that projects to the pupilloconstrictor nucleus may both be innervated by light sensitive retinal ganglion cells,(Lucas et al., 2001, 2003; Hattar et al., 2003; Barnard et al., 2006; Wong et al., 2007) we asked if an NMDA receptor could be demonstrated to be functionally active when the human pupillary light reflex is tested by a light flash.

Two NMDA antagonists, ketamine and nitrous oxide, are widely used as anesthetic agents and might block the light reflex if an NMDA receptor in the light reflex pathway is activated by light. However, when these agents are administered by themselves they often produce alterations in pupil size (White et al., 1982; Belani et al., 1993) and this confounds an assessment of drug effect on the light reflex because the dynamic properties of the iris are altered when pupil size changes (Loewenfeld, 1993). Furthermore, any change in pupil size alters the amount of light entering the pupil and this results in an altered stimulus.

Nitrous oxide and ketamine are also frequently used as adjuvants to vapor/opioid-based anesthetics and with this anesthetic regimen, pupil size is in a fixed miotic state (Larson et al., 1997; Larson and Talke, 2001). Previous studies have taken advantage of this unique property of opioid-based anesthetics to investigate the effect of drugs on the human pupillary light reflex (Larson and Talke, 2001). We thus used fentanyl to stabilize the size of the pupil and designed the following study to observe possible effects of ketamine and nitrous oxide on the light reflex. Our hypothesis was that nitrous oxide and ketamine would block the human pupillary light reflex when administered during desflurane/fentanyl anesthesia. We also

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measured systolic blood pressure, heart rate, and pupil size as other putative pharmacodynamic measures of these two drugs. Cortical function was measured with the bispectral index (BIS), a commonly used measure of anesthetic depth (Sebel et al., 1997).

## 2. Methods

After approval from the University of California Committee on Human Research and informed written consent, we studied 32 patients undergoing laparoscopic surgical procedures at Mt Zion Hospital, San Francisco. All were American Society of Anesthesiologists physical status 1 or 2, scheduled for procedures lasting over 3 h, free of eye disease, not taking opioids for chronic painful conditions, and not morbidly obese.

Midazolam (1–2 mg) was administered intravenously for sedation and after transfer to the operating theatre, anesthesia was induced with intravenous fentanyl 2 μg/kg and propofol, 1.5–3 mg/kg. Rocuronium bromide, 0.6–1 mg/kg, was administered to facilitate intubation of the trachea. Desflurane 4–5% end-tidal in 95% oxygen and fentanyl 2 μg/kg/h were administered to maintain anesthesia and intermittent boluses of rocuronium bromide were given to maintain muscular relaxation. The concentration of desflurane was adjusted between 4 and 5% end-tidal according to the clinical requirements. After the start of the study period, end-tidal concentrations of desflurane were kept constant and rocuronium bromide was administered to abolish the muscle twitch following stimulation of the ulnar nerve with surface electrodes (Digistim III, Neurotechnology, Houston, TX). Ventilation of the lungs of each patient was adjusted to maintain end-tidal $CO_2$ between 28 and 38 mm Hg. Body temperature was maintained between 35.5 and 37 °C using a forced air warming blanket ((Bair-Hugger Forced Air Warming Blanket, Arizant Healthcare, Eden Prairie, MN).

The study consisted of two parts. In the initial part 16 patients were randomized to receive either ketamine 1 mg/kg or saline as a bolus injection. Following this study, another randomized study of 16 patients was conducted in which patients received the addition of either 60% nitrogen or 60% nitrous oxide to the gas mixture. Randomization of study participants was performed by a draw from labeled cards. The investigator was blinded to the change in gas mixture (nitrogen or nitrous oxide) but was not blinded during the ketamine portion of the study. The patient was blinded during both arms of the study.

The study period was delayed until at least 1 h after the skin incision. After this time period, if the case was projected to last another 2 h, then the study proceeded. Prior to drug injections, the following parameters were measured and recorded every 2 min: systolic blood pressure, heart rate, BIS score, pupil size, pupillary light reflex, and end-tidal concentration of desflurane. After four such measures, separated by 2 min each, the study was commenced, but only if consistent stable measures were observed that were not increasing or decreasing from the prior measures by over 20%.

Following the baseline measurements, those selected for ketamine (or saline) were given the drug as a bolus diluted with saline to 10 $cm^3$ and administered over 15–30 s. Those selected to receive nitrous oxide (or nitrogen) were studied by setting the inspired mixture from 95% oxygen to 60% nitrous oxide or nitrogen and 40% oxygen. Adjustments of end-tidal desflurane were made each minute to keep desflurane concentrations constant during the following study period. Nitrous oxide or nitrogen was administered for 10 min and then the 6-liter gas flow was returned again to 95% oxygen. Measurements were continued every 2 min for 30 min after ketamine or saline injections and for 20 min after the start of nitrous oxide or nitrogen delivery. Nitrous oxide or nitrogen was therefore delivered for 10 min, followed by a 10 min recovery period.

Routine anesthetic monitors were used including continuous oxyhemoglobin saturation (Nellcor Oximax oxygen sensor, Tyco Healthcare Group, Pleasanton, CA). Noninvasive systolic arterial pressure and end-tidal gas analysis were measured by a S/5 Anesthesia Monitor using an ACX Photometer (Datex-Ohmeda, Inc., Madison, Wisconsin). Heart rate was recorded from the electrocardiograph. Direct measures of systolic pressures were used when an arterial catheter was in place. Core temperature was measured in the distal esophagus using an esophageal stethoscope (Respiratory Support Products, Inc, Irvine, CA). BIS scores were obtained from the Aspect XP Platform BIS monitor with electrodes designed for the BIS monitor.

The pupillary light reflex was measured using a portable infrared pupillometer (Neuroptics, Inc, Irvine, CA) (Du et al., 2005; Meeker et al., 2005) in a dark operating room. A flash of visible light with a wavelength of 550 nm, 800 ms duration, and intensity of 2300 lx was delivered at the start of each 3.2-s scan. The pupillometer calculates the reflex amplitude as the difference between the starting diameter and the minimum diameter obtained after the light flash. These reflex amplitudes were used to quantify the effect of ketamine and nitrous oxide on the light reflex. Only one measure of the pupil was taken at each two-minute interval and no measures were discarded or repeated. Pupillary measures were time stamped and stored in the pupillometer memory for later retrieval. Pupil size was the size of the pupil at the beginning of the 3.2-second scan. With a sample size of 8 subjects in each group we calculated that we could detect a 50% change in the light reflex with a power of 0.8 (with $P<.05$).

The averages of all four pre-drug measures were averaged and labeled "time zero" to form the baseline measure for calculations of areas above the curve (AAC). The AAC for the 30-minute duration following ketamine was calculated for each subject using the trapezoid rule and compared to the saline group with unpaired *t* tests. AAC and maximum change for the 2nd through the 12th minutes following nitrous oxide inhalation was calculated using the trapezoid rule and compared to the same time period for the nitrogen group using unpaired t tests. The maximum change, either positive or negative, for each parameter was called "maximum change". Time to maximum change was measured for all changes that were significantly different from the control group. $P<0.05$ was considered significant. Confidence intervals were calculated for all significant differences.

The effects of nitrous oxide and ketamine on the kinetic parameters of the light reflex were evaluated by downloading the scans onto Excel spreadsheets. We downloaded the scans for time points prior to drug administration, 10 min after the drug administration, and at the end of each study. These scans were then examined to measure latency of constriction, maximum amplitude of contraction, and 75% recovery time (time taken from the peak of the constriction response to obtain 75% recovery) (Prettyman et al., 1997). Repeated measures ANOVA with Bonferroni corrections were used to observe for differences in each kinetic parameter before the drug, 10 min after drug administration, and at the end of each study. Comparisons were made to the pre-drug measurements.

We then divided the scans into two phases in order to ascertain the part of the light reflex that was most affected by each drug. The first phase lasted from the start of the light reflex until the time of maximum constriction (constriction phase) and the second phase started when the pupil began to redilate and lasted until the end of the light reflex (dilation phase). We calculated the times to maximum effect on the reflex for both ketamine and nitrous oxide in order to ascertain whether the maximum effect occurred during the constriction phase or during the dilation phase. Paired t tests were used to compare time to maximum effect to time to maximum constriction. The Glantz statistical software program version 5.0 was used for the statistical calculations (Glantz, 2002).

## 3. Results

Demographic data and baseline measurements prior to drug administration are shown in Table 1. There were no differences in the age, weight or sex between the four groups. Baseline end-tidal