Supplemental Methods

Data Collection

We stored demographic, radiographic imaging, surgical, and outcomes data on the Research Electronic Data Capture (REDCap) software because it is (1) secure, (2) intuitive for users, (3) has audit capabilities to trace each step of data handling, and (4) has data export functions that can be used on common statistical packages (1). We collected medication, route of drug delivery as well as pupil observation data from medical records. The list of medication and route of drug delivery can be found in Supplementary Table 1.

We paired pupil measurements with all medications administered within 60 minutes prior to pupil measurement. We defined “medications administered” as a “new bag” or increase in dose, whereas the absence of a medication, static or decrease in dose was considered “not administered.” Our pupil measurements were paired with demographic, radiographic imaging, surgical, and outcomes data based on the study ID associated with the pupil measurement.

We created our primary outcome variables, average Neurologic Pupil Index™ (NPI) (2) and average resting pupil size, by taking the mean of the variables between the left and right eye. For our multivariable model, we accounted for the effect of ambient light conditions on pupil reactivity and size by creating a binary night-time indicator variable. To do this, we classified pupil measurements taken after 7:00 pm or before 7:00 am as the presence of night in our variable. To account for disease severity, we used the
ordinal Glasgow Coma Scale (GCS) score. We associated pupil measurements with GCS measured 60 minutes before or after pupil exam. Pupil measurements that did not fall under the 60 minutes time window were imputed with the last known GCS. To account for mass effect, we created a binary variable, where the presence of mass effect was considered if there was local displacement caused by some form of intracranial mass by expert review (HS, CJO).

**Analysis**

Rank Normalization

When we first examined the distribution of average NPi and average resting pupil size, we noticed that the data did not satisfy the normality assumption. To correct this, we performed a rank normalization, whereby values are associated with a rank along a normalized curve. To assess fit of our rank normalized data to a mixed effects linear regression, we graphically examined A) homoskedacity by plotting the rank normalized NPi against the residuals of our model (observed – expected based on our regression) and observing a random distribution of points, B) linearity by plotting the variance of the model residuals against the normalized NPi and observing equal variance across the outcome measurement, and C) normality by plotting the standardized residuals of the model against normalized quantiles and observing a linear relationship (Supplemental Figure 1).

**Patient – level Analysis**
We report p-values associated with our univariate analyses comparing baseline characteristics between patients with and without dexmedetomidine (student’s t test and chi square tests for continuous and categorical variables, respectively) in **Supplementary Table 2.**

**Pupil Observation – level Analysis**

Univariate analyses accounting for inter-patient correlation for **Figure 2** and **Supplementary Table 3** were calculated using multiple univariate mixed effects linear regressions where the exposure variable was a medication variable (binary) and the outcome variable was average NPi or average resting pupil size. We included a random effects term in our model to account for repeated observations from the same individuals. The median time between pupil observations was 123 minutes (IQR: 85-185). To assess significance of our primary objective (the effect of dexmedetomidine on average NPi and average resting pupil size), we included a Bonferroni correction ($\alpha=0.025$). Our model was constructed as follows: average NPi (or average resting pupil size) $\sim$ Primary medication exposure (dichotomous) + 1|Study ID + error.

Multivariable models for **Table 2** were calculated using multivariable mixed effects linear regression where the exposure variables included medication of interest (dexmedetomidine or acetaminophen), sex, age, GCS score, mass effect, acetaminophen, and night-indicator. Our outcome variable was either average NPi or average resting pupil size. Again, we included a random effects term to account for repeat observations. Our model was constructed as follows: average NPi (or average
resting pupil size) \sim Primary medication exposure (dichotomous) + sex + age + GCS + mass effect + acetaminophen + night-indicator + 1|Study ID + error.

We performed exploratory analysis comparing the effect of dexmedetomidine on NPi and resting pupil size in just the cohort of patients with at least one dose initiation or increase of dexmedetomidine (Supplementary Figure 2). We performed two multivariable mixed effects linear regression accounting for sex, age, GCS score, mass effect, acetaminophen, and night-indicator. These tests were performed similarly to the analysis done for Table 2. Our model was constructed as follows: average NPi (or average resting pupil size) \sim Primary medication exposure (dichotomous) + sex + age + GCS + mass effect + acetaminophen + night-indicator + 1|Study ID + error. We used the same multivariable model and subset of patient to explore the relationship between absolute dose of dexmedetomidine and NPi and resting pupil size for Supplementary Table 4. Our model was constructed as follows: average NPi (or average resting pupil size) \sim Primary medication exposure (absolute dose) + sex + age + GCS + mass effect + acetaminophen + night-indicator + 1|Study ID + error.

We also performed an exploratory subgroup analysis comparing the effect of dexmedetomidine on NPi and resting pupil size in the six most frequent primary diagnoses in our data set including spontaneous intraparenchymal hemorrhage, stroke, brain tumor, parenchymal traumatic brain injury, aneurysmal subarachnoid hemorrhage, and seizure (Supplemental Figure 3). We filtered patients by their primary diagnoses into subgroups and performed multiple independent univariate mixed effects linear
regression accounting for intra-patient correlation. These tests were performed using
the same statistical methods as analysis shown in Supplemental Table 3. Our model
was constructed as follows: average NPi (or average resting pupil size) ~ Primary
medication exposure (dichotomous) + 1|Study ID + error.

References
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Supplemental Figure 1. Graphical validation of rank-normalized average neurologic pupil index (NPI). We tested A) homoskedacity, B) linearity, and C) normality.

Supplemental Figure 2. Dexmedetomidine on pupil reactivity and size in patients with at least one dose of dexmedetomidine. Grouped boxplots showing the effect of dexmedetomidine on A) average NPI, and B) average resting pupil size between both eyes in patients with at least one dose initiation or increase during ICU stay. Displayed are boxes outlining the median, 25th, and 75th quartiles. Sample size presented shows the total number of patients and pupil observations associated with a dose increase. (+) dexmedetomidine represents an initiation or increase in dose, while (-) exposure represents absent, static, or decrease in dose. P values were calculated using multivariable mixed effects linear regression. Our model was constructed as follows: average NPI (or average resting pupil size) ~ Primary medication exposure (dichotomous) + sex + age + GCS + mass effect + acetaminophen + night-indicator + 1|Study ID + error. \( \alpha = 0.025^* \) due to Bonferroni. \( N \) = patients, and \( M \) = pupil observations.

Supplemental Figure 3. Dexmedetomidine on pupil reactivity and size across diagnostic groups. Grouped boxplots showing the effect of dexmedetomidine on A) average NPI, and B) average resting pupil size between both eyes in subgroups of patients with the most common diagnoses in our study cohort. Displayed are boxes outlining the median, 25th, and 75th quartiles. Sample sizes presented show the total
number of patients and pupil observations associated with the diagnosis. (+) dexmedetomidine represents an initiation or increase in dose, while (-) exposure represents absent, static, or decrease in dose. P values were calculated using univariate mixed effects linear regression. Our model was constructed as follows: average NPi (or average resting pupil size) ~ Primary medication exposure (dichotomous) + 1|Study ID + error. $\alpha=0.025^*$ due to Bonferroni. SAH = sub arachnoid hemorrhage, IPH = intra parenchymal hemorrhage, $N = \text{patients}$, and $M = \text{pupil observations}$. 
A) Homoskedasticity

B) Linearity

C) Normality
A) Dexmedetomidine and Pupil Reactivity Across Diagnostic Groups

B) Dexmedetomidine and Pupil Size Across Diagnostic Groups
### Supplementary Table 1. List of Drugs and Route of Delivery

| Medication Category | Route                      |
|---------------------|----------------------------|
| **IV Infusion Analgo-Sedatives** |                           |
| Dexmedetomidine     | Intravenous                |
| Propofol            | Intramuscular/Intravenous  |
| Fentanyl            | Intramuscular/Intravenous/Patch |
| Midazolam           | Intramuscular/Intravenous  |
| Ketamine            | Intramuscular/Intravenous  |
| Pentobarbital       | Intramuscular/Intravenous  |
| **Primary Sedatives** |                           |
| Clonidine           | Oral/Patch                 |
| Lorazepam           | Oral/Intramuscular/Intravenous |
| Clonazepam          | Oral                       |
| Phenobarbital       | Oral/Intramuscular/Intravenous |
| Clobazam            | Oral                       |
| Diazepam            | Oral/Intramuscular         |
| Trazodone           | Oral                       |
| Etomidate           | IM/Intravenous             |
| **Primary Analgesics** |                           |
| Acetaminophen       | Oral/Intravenous/Rectal    |
| Oxycodone           | Oral                       |
| Morphine            | Oral/Intramuscular/Intravenous |
| Meperidine          | Intravenous                |
| Gabapentin          | Oral                       |
| Hydromorphone       | Intramuscular/Intravenous  |
| Tramadol            | Oral                       |
| Hydrocodone         | Oral                       |
| Ibuprofen           | Oral                       |
Supplementary Table 2. P Values Associated with Baseline Characterization

| Variable                        | Total (N=221) | Dexmedetomidine (+) (N=37) | Dexmedetomidine (-) (N=184) | P Value |
|---------------------------------|---------------|-----------------------------|----------------------------|---------|
| **Demographics**                |               |                             |                            |         |
| Median Age (IQR) – yrs.         | 60 (50–68)    | 55 (44–60)                  | 61 (51.75–68)              | 0.016   |
| Male – no. (%)                  | 131 (59)      | 23 (62)                     | 108 (59)                   | 0.835   |
| Race – no. (%)                  |               |                             |                            |         |
| White                           | 153 (69)      | 22 (59)                     | 131 (71)                   | 0.126   |
| Black                           | 16 (7)        | 3 (8)                       | 13 (7)                     |         |
| Asian                           | 9 (4)         | 4 (11)                      | 5 (3)                      |         |
| Other¹                          | 43 (19)       | 8 (22)                      | 35 (19)                    |         |
| **Diagnosis**                   |               |                             |                            |         |
| Spontaneous IPH – no. (%)       | 49 (22)       | 4 (11)                      | 45 (24)                    | 0.001   |
| Stroke – no. (%)                | 49 (22)       | 5 (14)                      | 44 (24)                    |         |
| Brain Tumor – no. (%)           | 35 (16)       | 4 (11)                      | 31 (17)                    |         |
| Traumatic IPH – no. (%)         | 28 (13)       | 11 (30)                     | 17 (9)                     |         |
| Aneurysmal SAH – no. (%)        | 22 (10)       | 6 (16)                      | 16 (9)                     |         |
| Seizure – no. (%)               | 13 (6)        | 5 (14)                      | 8 (4)                      |         |
| Other² – no. (%)                | 25 (11)       | 2 (5)                       | 23 (12)                    |         |
| **Markers of Disease Severity** |               |                             |                            |         |
| Mass Effect – no. (%)           | 162 (73)      | 26 (70)                     | 136 (74)                   | 0.800   |
| Midline Shift – no. (%)         | 112 (51)      | 18 (49)                     | 94 (51)                    | 0.928   |
| Uncal Herniation – no. (%)      | 72 (33)       | 9 (24)                      | 63 (34)                    | 0.326   |
| Intracranial Pressure Monitor – no. (%) | 78 (35) | 18 (49) | 60 (33) | 0.094 |
| External Ventricular Drain – no. (%) | 24 (11) | 7 (19) | 17 (9) | 0.151 |
| Craniectomy – no. (%)           | 36 (16)       | 8 (22)                      | 26 (15)                    | 0.472   |
| Mechanical Ventilation – no. (%)| 172 (78)      | 37 (100)                    | 135 (73)                   | 0.001   |
| Death at Discharge – no. (%)    | 74 (33)       | 4 (11)                      | 70 (38)                    | 0.003   |
| **Analogo-sedative Medications**|               |                             |                            |         |
| IV Analogosedative Infusions – no. (%) | 123 (56) | 37 (100) | 86 (47) | <0.001 |
| Primary Sedatives – no. (%)     | 49 (22)       | 21 (57)                     | 28 (15)                    | <0.001  |
| Primary Analgesics – no. (%)    | 162 (73)      | 35 (95)                     | 127 (69)                   | 0.003   |

IQR = inter quartile range, SAH = sub arachnoid hemorrhage, IPH = intra parenchymal hemorrhage, IV = intravenous, and N = patients

(+): dexmedetomidine represents an initiation or increase in dose, while (-): dexmedetomidine represents absent, static, or decrease in dose.
P-values were calculated by Student’s t test and chi square test for continuous or categorical variables, respectively.

¹Other races: Native American (N=1), and Unspecified (N=42).

²Other diagnoses: Cerebral Sinus Venous Thrombosis (N=4), Non-Aneurysmal Sub Arachnoid Hemorrhage (N=1), Epidural Hemorrhage (N=1), Sub Dural Hemorrhage (N=6), Isolated Interventricular Hemorrhage (N=1), Isolated Hydrocephalus (N=2), Infection (N=5), Moya Moya Disease (N=1), MCA Aneurysm (N=1), Anti-NMDA Encephalitis (N=1), Autoimmune Encephalitis (N=1), and Posterior Reversible Encephalopathy Syndrome (N=1).
### Supplementary Table 3. Univariate Models of Analgo-Sedatives on Pupil Reactivity and Size

| Medication                          | NPI (+)     | NPI (-)† | P Value  | RPS-mm (+)  | RPS-mm (-)† | P Value  |
|------------------------------------|-------------|----------|----------|-------------|-------------|----------|
| **IV Analgo-Sedative Infusions**   |             |          |          |             |             |          |
| Dexmedetomidine†                   | 3.92 ± 1.10 | 3.76 ± 1.22 | <0.001  | 3.16 ± 1.03 | 3.43 ± 1.13 | <0.001  |
| Propofol                           | 4.14 ± 0.87 | 3.77 ± 1.22 | <0.001† | 3.13 ± 0.96 | 3.41 ± 1.13 | <0.001† |
| Fentanyl                           | 3.99 ± 1.03 | 3.77 ± 1.22 | 0.048    | 3.19 ± 1.04 | 3.41 ± 1.13 | <0.001  |
| Midazolam                          | 3.77 ± 1.26 | 3.78 ± 1.21 | 0.041    | 3.15 ± 1.15 | 3.41 ± 1.12 | <0.001  |
| Ketamine                           | 3.81 ± 1.22 | 3.78 ± 1.22 | 0.134    | 2.71 ± 0.74 | 3.41 ± 1.13 | <0.001  |
| Pentobarbital                      | 3.12 ± 0.91 | 3.78 ± 1.22 | 0.692    | 4.20 ± 1.77 | 3.40 ± 1.13 | 0.576   |
| Clobazam                           | 1.68 ± 1.64 | 3.78 ± 1.21 | 0.397    | 3.39 ± 0.38 | 3.40 ± 1.13 | 0.150   |
| **Primary Sedative**               |             |          |          |             |             |          |
| Clonidine                          | 3.87 ± 1.04 | 3.78 ± 1.22 | 0.150    | 3.84 ± 1.18 | 3.39 ± 1.12 | <0.001  |
| Lorazepam                          | 3.71 ± 1.08 | 3.78 ± 1.22 | 0.628    | 3.99 ± 1.15 | 3.40 ± 1.13 | <0.001  |
| Clonazepam                         | 3.86 ± 1.21 | 3.78 ± 1.22 | 0.005    | 3.62 ± 1.18 | 3.40 ± 1.12 | 0.001   |
| Phenobarbital                      | 4.29 ± 0.40 | 3.78 ± 1.22 | 0.502    | 4.21 ± 1.09 | 3.40 ± 1.12 | 0.913   |
| Clobazam                           | 4.29 ± 0.48 | 3.78 ± 1.22 | 0.039    | 3.12 ± 0.87 | 3.40 ± 1.13 | 0.914   |
| Diazepam                           | 3.16 ± 1.42 | 3.78 ± 1.21 | 0.007    | 4.30 ± 1.22 | 3.40 ± 1.12 | <0.001  |
| Tramadol                           | 4.07 ± 0.40 | 3.78 ± 1.22 | 0.951    | 3.18 ± 1.23 | 3.40 ± 1.13 | 0.616   |
| Trazodone                          | 4.60 ± 0.14 | 3.78 ± 1.22 | 0.064    | 3.56 ± 1.27 | 3.40 ± 1.13 | 0.390   |
| Etomidate                          | 3.25        | 3.78 ± 1.22 | 0.672    | 2.79        | 3.40 ± 1.13 | 0.637   |
| **Primary Analgesic**              |             |          |          |             |             |          |
| Acetaminophen                      | 4.00 ± 0.95 | 3.74 ± 1.25 | 0.001    | 3.35 ± 1.07 | 3.41 ± 1.13 | 0.123   |
| Oxycodone                          | 4.02 ± 0.94 | 3.75 ± 1.24 | 0.004    | 3.35 ± 1.07 | 3.41 ± 1.13 | 0.203   |
| Morphine                           | 3.91 ± 0.92 | 3.77 ± 1.22 | 0.241    | 3.51 ± 1.05 | 3.40 ± 1.13 | 0.034   |
| Meperidine                         | 4.01 ± 0.94 | 3.77 ± 1.22 | 0.107    | 3.44 ± 1.19 | 3.40 ± 1.13 | 0.467   |
| Gabapentin                         | 4.21 ± 0.58 | 3.77 ± 1.22 | 0.217    | 2.98 ± 0.91 | 3.41 ± 1.13 | 0.007   |
| Hydromorphone                      | 3.53 ± 1.68 | 3.78 ± 1.21 | 0.070    | 3.13 ± 1.08 | 3.40 ± 1.13 | 0.100   |
| Tramadol                           | 4.68 ± 0.12 | 3.78 ± 1.23 | 0.030    | 3.79 ± 0.75 | 3.40 ± 1.13 | 0.621   |
| Ibuprofen                          | 3.55 ± 0.35 | 3.78 ± 1.22 | 0.228    | 5.75 ± 0.66 | 3.40 ± 1.13 | 0.428   |
| NPI = neurologic pupil index, RPS = resting pupil size, IV = intravenous, N = patients, and M = pupil observations.  
(+/-) = dexmedetomidine represents an initiation or increase in dose, while (-) = dexmedetomidine represents absent, static, or decrease in dose.  
Displayed is the mean ± standard deviation.  
Sample sizes presented show the total number of patients and pupil observations associated medication.  
P values were calculated using univariate mixed effects linear regression. Our model was constructed as follows: average NPi (or average resting pupil size) ~ Primary medication exposure (dichotomous) + 1|Study ID + error.  
†Primary hypothesis.  
†NPi and RPS values are very similar in (-) groups due to the large amounts of observations (between 8913 and 9896) in the (-) groups compared to the (+) groups.
## Supplementary Table 4. Multivariable Model of Dexmedetomidine Dose on Pupil Reactivity and Size

| Dexmedetomidine Model                  | Neurologic Pupil Index β | P Value | Resting Pupil Size β | P Value |
|---------------------------------------|--------------------------|---------|----------------------|---------|
| Dexmedetomidine Dose (N=37, M=166)    | 0.01 ± 0.03              | 0.74    | -0.0003 ± 0.05       | 0.99    |
| Male                                  | 0.45 ± 0.27              | 0.09    | -0.09 ± 0.17         | 0.60    |
| Age – yrs.                            | 0.01 ± 0.008             | 0.07    | -0.01 ± 0.005        | 0.03    |
| Glasgow Coma Score                    | 0.03 ± 0.005             | <0.001  | 0.05 ± 0.007         | <0.001  |
| Mass Effect                           | -0.40 ± 0.27             | 0.14    | -0.15 ± 0.17         | 0.37    |
| Acetaminophen                          | 0.05 ± 0.03              | 0.10    | 0.04 ± 0.04          | 0.24    |
| Night-time                            | 0.16 ± 0.02              | <0.001  | 0.12 ± 0.03          | <0.001  |

\(N=\) patients, and \(M=\) pupil observations. 

\(\beta\) coefficients represent the change in rank-normalized units of the outcome with a 1 mg/kg/hr increase in dexmedetomidine dose, the presence of a dichotomous variable (male, mass effect, acetaminophen, night-time variable), and an increase in continuous or ordinal scale (age and Glasgow Coma Score).

Sample sizes presented show the total number of patients and pupil observations associated with an initiation or an increase in dexmedetomidine dose less than 60 minutes prior to pupil examination.

P values were calculated using multivariable mixed effects linear regression. Our model was constructed as follows: average NPi (or average resting pupil size) ~ Dexmedetomidine dose + sex + age + GCS + mass effect + acetaminophen + night-indicator + 1|Study ID + error.