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 (3). 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 (4). 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)** (5).

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 (α=0.025). Our model was constructed as follows: average NPi (or average resting pupil size) ~ 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) ~ 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) ~ 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) ~ 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

1. Harris PA, Taylor R, Thielke R, et al.: Research electronic data capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009; 42:377–381

2. Olson DM, Stutzman SE, Atem F, et al.: Establishing Normative Data for Pupillometer Assessment in Neuroscience Intensive Care: The “END-PANIC” Registry. *J Neurosci Nurs* 2017; 49:251–254

3. Teasdale G, Jennett B: ASSESSMENT OF COMA AND IMPAIRED CONSCIOUSNESS. *The Lancet* 1974; 304:81–84

4. Qiu X, Wu H, Hu R: The impact of quantile and rank normalization procedures on the testing power of gene differential expression analysis. *BMC Bioinformatics* 2013; 14:124

5. Harrison XA, Donaldson L, Correa-Cano ME, et al.: A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ* 2018; 6:e4794

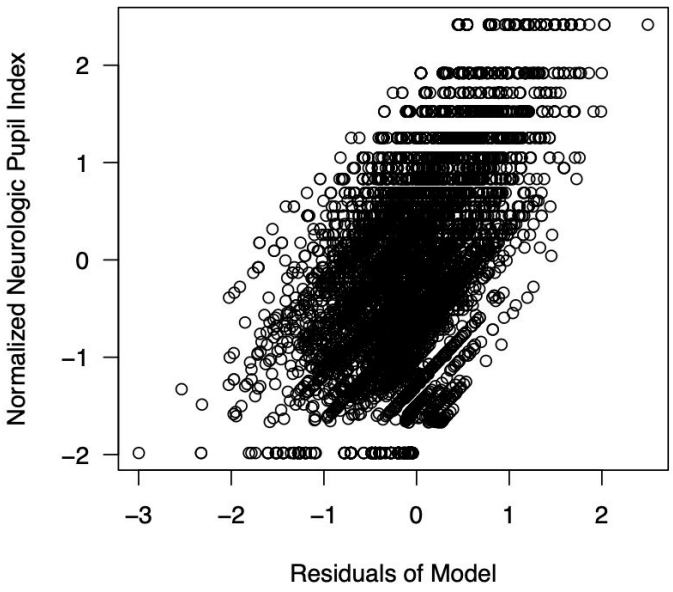
Supplemental Figure 1. Graphical validation of rank-normalized average neurologic pupil index (NPi). We tested **A)** homoskedacity, **B)** linearity, and **C)** normality.

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. α=0.025* due to Bonferroni. *N* = patients, and *M* = pupil observations.

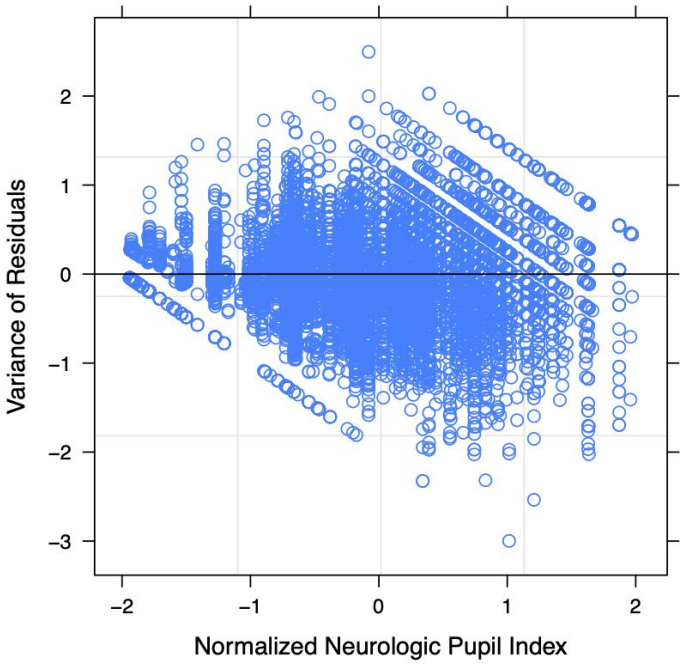
Supplemental Figure 3. Dexmedetomidine on pupil reactivity and size across diagnostic groups. Grouped boxplots showing the effect of dexmedetomidine on **A)** average NPi, and **B)** average resting pupil size between both eyes in subgroups of patients with the most common diagnoses in our study cohort. 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. α=0.025* due to Bonferroni. SAH = sub arachnoid hemorrhage, IPH = intra parenchymal hemorrhage, *N* = patients, and *M* = pupil observations.

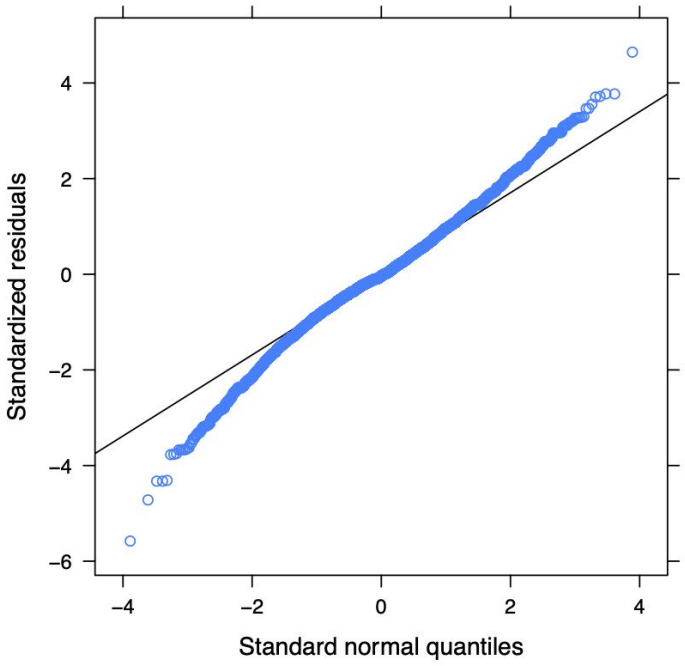
A) Homoskedacity

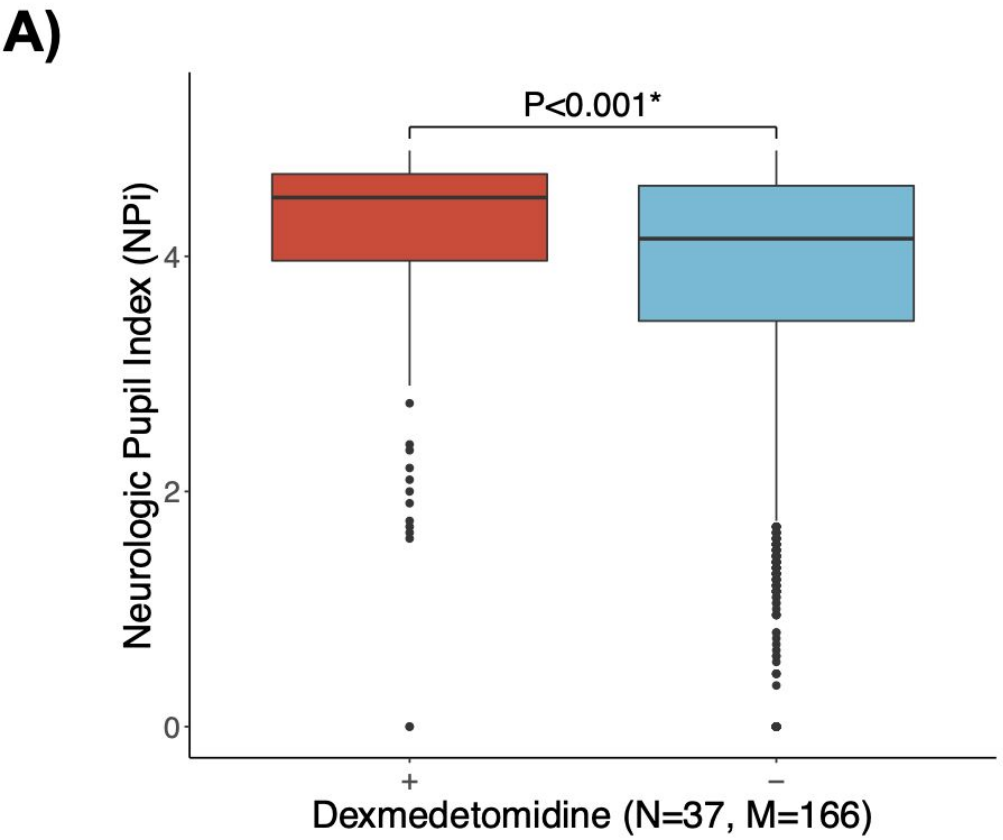


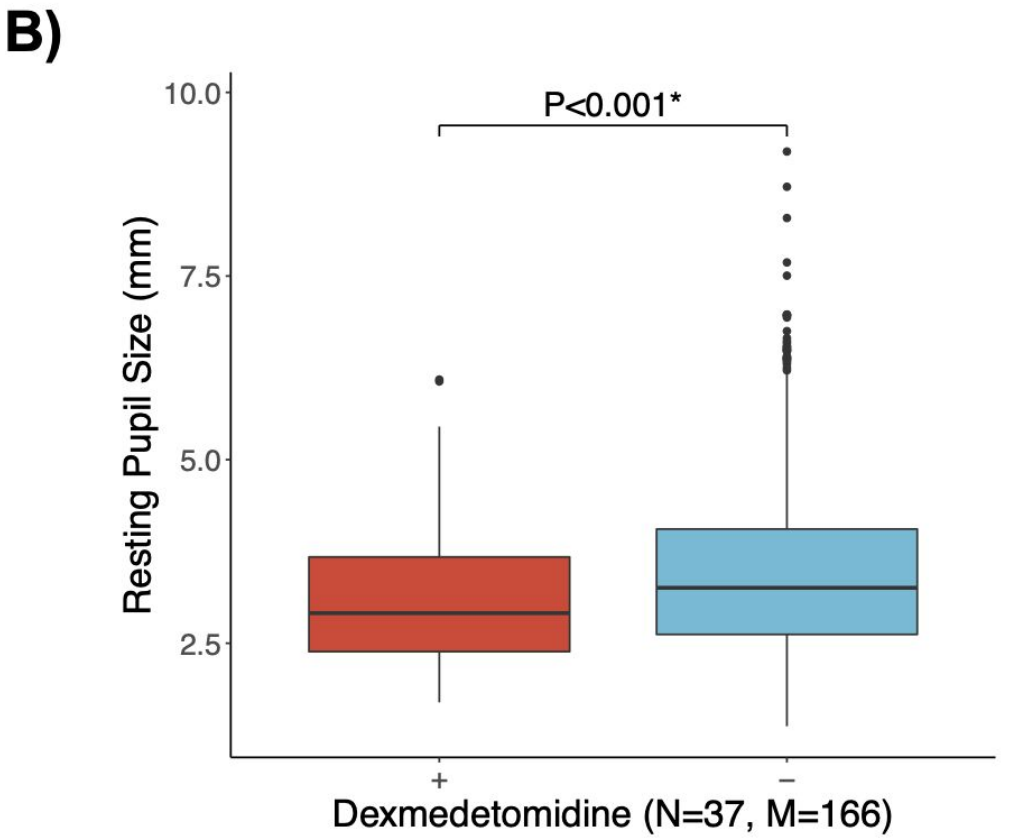
B) Linearity



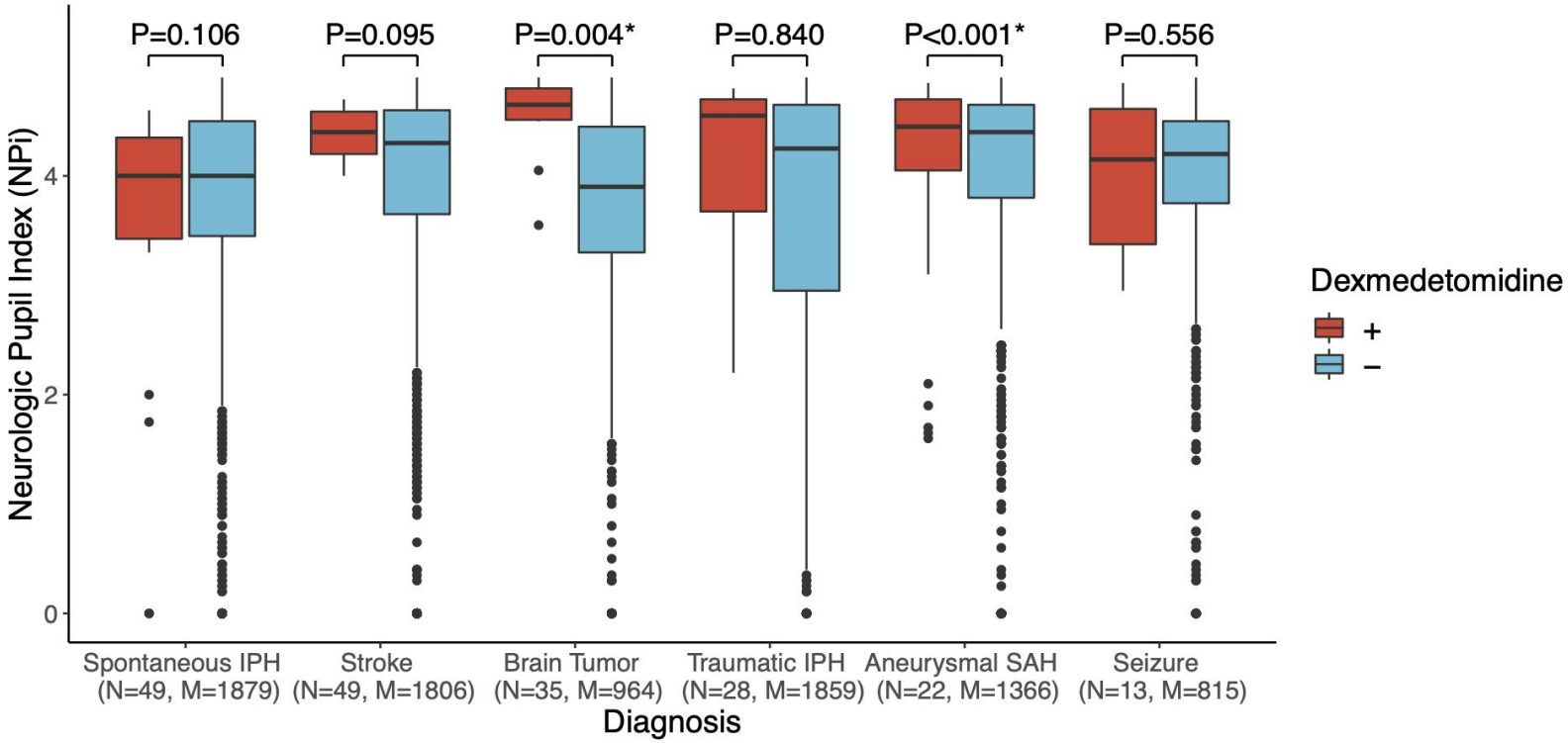
C) Normality



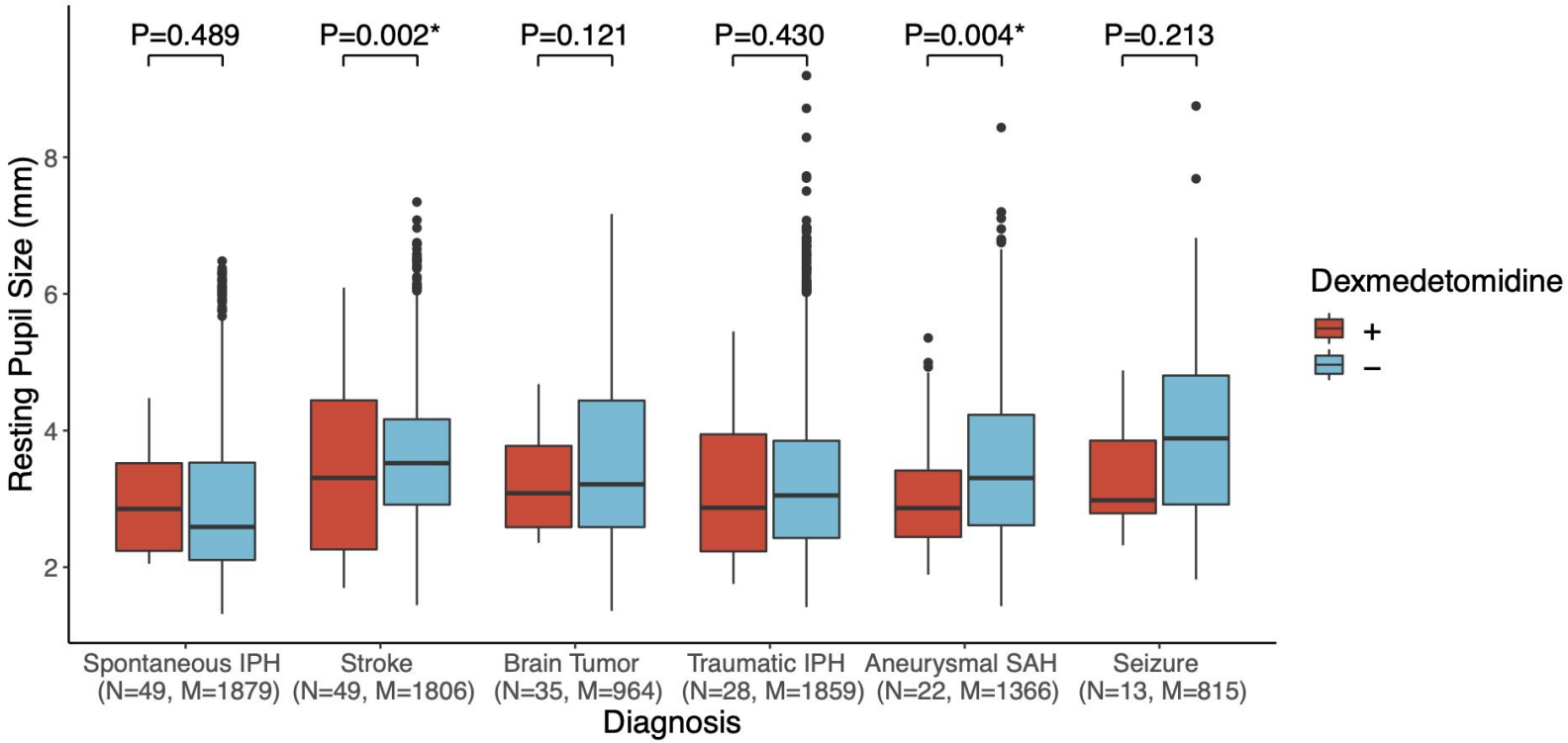

A)
P<0.001*
Neurologic Pupil Index (NPi)
0
2
4
+
−
Dexmedetomidine (N=37, M=166)


B)
P<0.001*
Resting Pupil Size (mm)
2.5
5.0
7.5
10.0
+
−
Dexmedetomidine (N=37, M=166)

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)	
Other[1]	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)	
Other[2] – 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)	28 (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
Analgo-sedative Medications				
IV Analgo-Sedative 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.

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

[2]Other 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 (-)[1]	P Value	RPS–mm (+)	RPS–mm (-)[1]	P Value
IV Analgo-Sedative Infusions		3.92 ± 1.10	3.76 ± 1.22	<0.001	3.16 ± 1.03	3.43 ± 1.13	<0.001
Dexmedetomidine†	(N=37, M=166)	4.14 ± 0.87	3.77 ± 1.22	<0.001†	3.13 ± 0.96	3.41 ± 1.13	<0.001†
Propofol	(N=106, M=479)	3.99 ± 1.03	3.77 ± 1.22	0.048	3.19 ± 1.04	3.41 ± 1.13	<0.001
Fentanyl	(N=68, M=231)	3.77 ± 1.26	3.78 ± 1.21	0.041	3.15 ± 1.15	3.41 ± 1.12	<0.001
Midazolam	(N=16, M=49)	3.81 ± 1.22	3.78 ± 1.22	0.134	2.71 ± 0.74	3.41 ± 1.13	<0.001
Ketamine	(N=2, M=4)	3.12 ± 0.91	3.78 ± 1.22	0.692	4.20 ± 1.77	3.40 ± 1.13	0.576
Pentobarbital	(N=3, M=5)	1.68 ± 1.64	3.78 ± 1.21	0.397	3.39 ± 0.38	3.40 ± 1.13	0.150
Primary Sedative		3.87 ± 1.04	3.78 ± 1.22	0.150	3.84 ± 1.18	3.39 ± 1.12	<0.001
Clonidine	(N=23, M=89)	3.71 ± 1.08	3.78 ± 1.22	0.628	3.99 ± 1.15	3.40 ± 1.13	<0.001
Lorazepam	(N=19, M=52)	3.86 ± 1.21	3.78 ± 1.22	0.005	3.62 ± 1.18	3.40 ± 1.12	0.001
Clonazepam	(N=2, M=53)	4.29 ± 0.40	3.78 ± 1.22	0.502	4.21 ± 1.09	3.40 ± 1.12	0.913
Phenobarbital	(N=8, M=41)	4.29 ± 0.48	3.78 ± 1.22	0.039	3.12 ± 0.87	3.40 ± 1.13	0.914
Clobazam	(N=7, M=31)	3.16 ± 1.42	3.78 ± 1.21	0.007	4.30 ± 1.22	3.40 ± 1.12	<0.001
Diazepam	(N=3, M=9)	4.07 ± 0.40	3.78 ± 1.22	0.951	3.18 ± 1.23	3.40 ± 1.13	0.616
Trazodone	(N=1, M=2)	4.60 ± 0.14	3.78 ± 1.22	0.064	3.56 ± 1.27	3.40 ± 1.13	0.390
Etomidate	(N=1, M=1)	3.25	3.78 ± 1.22	0.672	2.79	3.40 ± 1.13	0.637
Primary Analgesic		4.00 ± 0.95	3.74 ± 1.25	0.001	3.35 ± 1.07	3.41 ± 1.13	0.123
Acetaminophen	(N=139, M=984)	4.02 ± 0.94	3.75 ± 1.24	0.004	3.35 ± 1.07	3.41 ± 1.13	0.203
Oxycodone	(N=53, M=226)	3.91 ± 0.92	3.77 ± 1.22	0.241	3.51 ± 1.05	3.40 ± 1.13	0.034
Morphine	(N=40, M=156)	4.01 ± 0.94	3.77 ± 1.22	0.107	3.44 ± 1.19	3.40 ± 1.13	0.467
Meperidine	(N=13, M=114)	4.21 ± 0.58	3.77 ± 1.22	0.217	2.98 ± 0.91	3.41 ± 1.13	0.007
Gabapentin	(N=20, M=77)	4.30 ± 0.48	3.77 ± 1.22	0.450	3.34 ± 1.20	3.40 ± 1.13	0.030
Hydromorphone	(N=18, M=43)	3.53 ± 1.68	3.78 ± 1.21	0.070	3.13 ± 1.08	3.40 ± 1.13	0.100
Tramadol	(N=4, M=8)	4.68 ± 0.12	3.78 ± 1.23	0.050	3.79 ± 0.75	3.40 ± 1.13	0.621
Hydrocodone	(N=1, M=2)	3.55 ± 0.35	3.78 ± 1.22	0.228	5.75 ± 0.66	3.40 ± 1.13	0.428
Ibuprofen	(N=1, M=1)	1.70	3.78 ± 1.21	0.912	5.08	3.40 ± 1.13	0.665

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.
[1]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.
β 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.