List of abbreviations / glossary

HIT	High Impact Trauma (i.e., strikes)
Anesthetic Conditions / Interventions (10)	
postHYPR	Hyperoxia only, after strikes
postHYPRiso	Hyperoxia + Isoflurane, administered after strikes
postHYPRsevo	Hyperoxia + Sevoflurane, administered after strikes
postISO	Isoflurane in normoxia only, administered after strikes
postSEVO	Sevoflurane in normoxia only, administered after strikes
preHYPR	Hyperoxia only, before strikes
preHYPRiso	Hyperoxia + Isoflurane, administered before strikes
preHYPRsevo	Hyperoxia + Sevoflurane, administered before strikes
preISO	Isoflurane in normoxia only, administered before strikes
preSEVO	Sevoflurane in normoxia only, administered before strikes
Treatments	
C	Control; normoxia, no strikes nor any of above interventions
H	Only strikes (HIT), normoxia and no interventions
GA	Only anesthetic / intervention, no strikes
GAH	Anesthetic / intervention, plus strikes

Methods

Data collected from each vial consisted of the number of flies alive at the start of an experimental trial (m) and the number of flies that were dead at a trial's conclusion (y). Log-binomial regression was used to estimate and test whether mortality (y/m) differed as a function of anesthesia, hit, or the interaction between these two factors, and whether any of these elements differed among the 10 conditions defined by combinations of anesthesia (ISO, SEVO, or none; each with and without hyperoxia) and timing (pre- vs post-administration). These models were separately fit to each age group (young vs old). For experimental conditions where mortality was especially low ($y \ll m$), Poisson regression— with y as the response and $\log(m)$ as an offset—was instead used to estimate mortality rate. Standard errors for these models were computed using a sandwich estimator of variance to account for having used an alternate distribution (i.e., Poisson approximation to binomial for rare events). Statistical significance was set to 0.05 with supporting 95% confidence intervals for ratios involving mortality risk. All analyses were performed using R (v. 3.5.1)[1] and the accompanying *sandwich* package.[2]

Two Strikes

Design

The number of vials under each combination of experimental conditions is given in the table below. Anesthesia and high-impact trauma (HIT) were applied to vials and therefore serves as the fundamental unit of analysis.

Table 1. Number of vials for each condition (row), age group (1--8 days or 43--50 days) and treatment (*C*=control; *GA*=anesthesia only; *GAH*=both anesthesia and HIT; *H*=HIT only).

	Age group (2 levels) and Treatment (4 levels)							
	1 to 8 days				43 to 50 days			
Condition	C	GA	GAH	H	C	GA	GAH	H
postHYPR	14	14	14	14	14	14	14	14
postHYPRiso	14	14	14	14	14	14	14	14
postHYPRsevo	16	16	16	16	16	16	16	16
postISO	16	16	16	16	16	16	16	16
postSEVO	12	12	12	12	12	12	12	12
preHYPR	12	12	12	12	12	12	12	12
preHYPRiso	14	14	14	14	14	14	14	14
preHYPRsevo	14	14	14	14	14	14	14	14
preISO	12	12	12	12	12	12	12	12
preSEVO	12	12	12	12	12	12	12	12

Results

The full experiment consisted of 1,088 vials distributed among 80 distinct combinations of condition, age, and treatment. Each combination involved either 12 (40% of experiments), 14 (40%), or 16 (20%) vials, with each vial containing anywhere from $m = 10$–60 live flies initially: $m = 20$ per vial was most prevalent (49.1%), followed by $m = 25$ (27.4%) and $m = 19$ (5.1%).

[1] R Core Team. (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

[2] Zeileis, A. (2006). Object-oriented computation of sandwich estimators. *Journal of Statistical Software*, 16(9):1–16. URL:http://doi.org/10.18637/jss.v016.i09.

Young (Age 1–8 days)

No HIT; *C* and *GA* Mortality rate for controls did not differ among the 10 conditions determined by anesthetic type and timing ($X^2_9 = 6.486,\ p = 0.690$) and was estimated to be 0.618 (95% CI: 0.161–2.38) deaths per 1000 flies. Neither was there any association between mortality and condition in the pure general anesthesia treatment (*GA*; $X^2_9 = 10.28,\ p = 0.328$), where mortality was 4.33 (95% CI: 2.57–7.30) per 1000 flies. This translates to a 7-fold (95% CI: 1.63–30 fold; $p = 0.01$) increase in mortality risk for *GA* relative to *C*, and represents an absolute increase of 3.7 (95% CI: 1.3–6.7) additional deaths per 1000 flies (when *GA* is present vs not).

HIT; *H* and *GAH* No evidence ($X^2_9 = 5.46,\ p = 0.793$) was found to indicate any significant differences among the 10 conditions for flies exposed only to hits. Overall, mortality for flies within this age group exposed solely to HIT is estimated to be 13.7 (95% CI: 12.6–14.9) deaths per 100 flies.

Table 2. Estimated mortality rate (death per **100** flies) for GAH and H (constant), by condition. Fold changes (GAH/H) are given with p-value testing whether actual fold change deviates from 1.

	GAH	H	Fold-change (GAH/H)	
Condition	est. (95% CI)	est. (95% CI)	est. (95% CI)	p-value
postHYPR	15.7 (12.0, 20.6)	13.7 (12.6, 14.9)	1.15 (0.87, 1.53)	0.334
postHYPRiso	18.1 (14.3, 22.9)	13.7 (12.6, 14.9)	**1.32 (1.03, 1.70)**	**0.029**
postHYPRsevo	14.4 (11.0, 18.8)	13.7 (12.6, 14.9)	1.05 (0.79, 1.39)	0.723
postISO	17.7 (14.1, 22.2)	13.7 (12.6, 14.9)	**1.30 (1.02, 1.65)**	**0.035**
postSEVO	16.7 (12.6, 22.1)	13.7 (12.6, 14.9)	1.22 (0.91, 1.64)	0.187
preHYPR	11.0 (8.0, 15.2)	13.7 (12.6, 14.9)	0.80 (0.58, 1.12)	0.201
preHYPRiso	6.9 (4.7, 10.1)	13.7 (12.6, 14.9)	**0.50 (0.34, 0.74)**	**0.001**
preHYPRsevo	8.0 (5.6, 11.4)	13.7 (12.6, 14.9)	**0.59 (0.41, 0.84)**	**0.004**
preISO	7.5 (5.7, 9.9)	13.7 (12.6, 14.9)	**0.55 (0.42, 0.73)**	**<0.001**
preSEVO	6.7 (4.5, 9.8)	13.7 (12.6, 14.9)	**0.49 (0.33, 0.72)**	**<0.001**

It should be noted that within *GAH* there was no indication of differences among the five 'Post' conditions ($X^2_4 = 2.12,\ p = 0.713$) nor any difference among the five 'Pre' conditions ($X^2_4 = 5.01,\ p = 0.286$). That is, the 20 separate combinations involving two treatments (*GAH* & *H*) and 10 conditions can be resolved to just three distinct groups with no appreciable loss of information ($X^2_{17} = 12.60,\ p = 0.763$).

Table 3. Estimated mortality rate (deaths per 100 flies) for GAH and H (constant), by condition (Post- and Pre-exposures). Fold changes (GAH/H) are given with p-value testing whether actual fold change deviates from 1.

	GAH	H	Fold-change (GAH/H)	
	Est. (95% CI)	Est. (95% CI)	Est. (95% CI)	p-value
Post-exposure	16.5 (14.8, 18.5)	13.7 (12.6, 14.9)	1.21 (1.05, 1.39)	0.008
Pre -exposure	7.9 (6.8, 9.1)	13.7 (12.6, 14.9)	0.58 (0.48, 0.68)	<0.001

In absolute terms, the Post-exposure response for *GAH* has an excess of 2.87 (95% CI: 0.72–5.12) deaths per 100 flies as compared to the Post-exposure response for *H*, while the Pre-exposure response has 5.8 (95% CI: 4.12–7.41) fewer deaths per 100 flies for *GAH* compared to *H*. When exposed to both hits and anesthetics, the Post-exposure conditions typically have 8.67 (95% CI: 6.47–10.92) additional deaths per 100 flies than the Pre-exposure conditions.

Table 4 provides mortality estimates (deaths per 1000 flies exposed) for all conditions and treatments for the Age 1–8 day group.

Four specific comparisons within *GAH* Special attention was given to the effect of hyperoxia in both pre- and post-exposure to each of the anesthetics (ISO & SEVO). This effect was calculated from relevant groups within *GAH* treatment, with and without hyperoxia, while holding the other two elements (timing of exposure, anesthetic) constant. None of these comparisons was significant.

Table 5. Estimated fold changes and 95% CIs for pre-selected comparisons of interest; p-values test whether the actual fold change differs from unity.

	Fold-change within GAH	
Comparison	est. (95% CI)	p-value
preISO / preHYPRiso	1.10 (0.69, 1.77)	0.691
preSEVO / preHYPRsevo	0.83 (0.43, 1.41)	0.496
postISO / postHYPRiso	0.98 (0.71, 1.36)	0.907
postSEVO/ postHYPRsevo	1.16 (0.79, 1.71)	0.456

Old (Age 43–50 days)

No anesthetic; *C* and *H* Mortality rate for controls (*C*) does not remain constant over the ten conditions ($X_9 = 58.7,\ p < 0.001$) and appears instead to segregate into three distinct sets. Four of the five post-exposure conditions (postHYPR, postHYPRsevo, postISO, postSEVO) share a common mortality rate of 1.46 (95% CI: 0.85–2.49) deaths per 100 flies. Both of the pre-exposure conditions without HYPR have the highest mortality rate of 5.66 (95% CI: 3.86–8.30) deaths per 100 flies. The lowest rate is shared by postHYPRiso, preHYPR, preHYPRiso, and preHYPRsevo and is estimated to be 0.33 (95% CI: 0.11–0.96) deaths per 100 flies. This simplification from 10 separate mortality rates to just three rates shared among select conditions does not lead to a loss of information ($X_7^2 = 6.58,\ p = 0.474$).

The mortality rate among flies exposed only to HIT did not significantly vary among conditions ($X_9^2 = 8.47,\ p = 0.487$) and was estimated to be 43.8 (95% CI: 42.1–45.6) deaths per 100 flies overall (shared by all conditions).

Anesthetic ; *GA* and *GAH* Treatments *GA* and *GAH* each showed evidence of overdispersion, with variation in mortality rate approximately 1.3 and 1.7 times that expected from genuine binomial data. Log-binomial models for these two treatments had standard errors inflated to account for this extra variation and subsequent testing was based on the F-distribution (rather than X^2). These adjustments lead to slightly wider confidence intervals and more conservative p-values when testing for significance. Even after these corrections, mortality rate was still found to significantly differ among the 10 conditions for *GA* treatment ($F_{9,126} = 5.32,\ p < 0.001$) as well as the *GAH* treatment ($F_{9,126} = 2.64,\ p = 0.008$) with no discernable pattern for how rates within a treatment might be grouped or simplified. The full set of mortality estimates as well as general comparisons for *GAH* relative to *GA* are given in Table 6.

Four specific comparisons within *GAH* Special attention was given to the effect of hyperoxia in both pre- and post-exposure to each of the anesthetics (ISO & SEVO). This effect was calculated from relevant groups within *GAH* treatment, with and without hyperoxia, while holding the other two elements (timing of exposure, anesthetic) constant.

Table 7. Estimated fold changes and 95% CIs for pre-selected comparisons of interest; p-values test whether the actual fold change differs from unity.

Comparison	Fold-change within GAH est. (95% CI)	p-value
preISO / preHYPRiso	0.93 (0.76, 1.15)	0.514
preSEVO / preHYPRsevo	1.02 (0.80, 1.28)	0.894
postISO / postHYPRiso	**0.81 (0.67, 0.97)**	**0.023**
postSEVO/ postHYPRsevo	1.06 (0.87, 1.31)	0.552

Mortality rate for postISO (≈ 49.9%) is 19% (95% CI: 3–33%) lower than the mortality rate for postHYPRiso (≈ 61.7%). Three other comparisons have relative rates that don't strongly deviate from one.

Four Strikes

Design

A subset of original conditions and treatments were analyzed for vials of flies exposed to four strikes, with between 16–28 vials per combination of condition and treatment. Each vial usually contained either 20, 25, 40, or 50 flies (70% of all configurations) and no experiment used fewer than 19 flies per vial.

Table 8. Design layout for the four-strike experiment showing number of vials used for each combinations of condition and treatment involving a given age group.

Condition	1-8 days GAH	1-8 days H	29-36 days GAH	29-36 days H	43-50 days GAH	43-50 days H
TBI				26		24
postISO	22	22				
postSEVO	12	12				
preISO	28	28				
preSEVO	16	16				

Results

Overdispersion in mortality rates was evident in flies exposed to four strikes, and once again the expected variance from the log-binomial models was inflated (by a factor of 1.5–1.8, depending on condition/treatment) for all tests and construction of confidence intervals; test statistics and statistical significance is based on the F-distribution. Estimates of mortality (as a percent) corresponding to TBI (i.e., only high-impact trauma) are given below. Information pertaining to two strikes is taken from Tables 4 & 6 from the previous section and is visualized in Figure 1 of the main article.

Table 9. Percent mortality for flies exposed to either 2 or 4 strikes, separated by age. Information pertaining to 2 strikes is taken from earlier Tables 4 & 6.

Age (days)	Strikes	Condition	Mortality (%) and 95% CI
1--8	2	TBI (n=136)	13.7 (12.6, 14.9)
	4	PostISO (n=22)	23.5 (20.3, 27.4)
		PostSEVO, PreISO PreSEVO (n=56)	33.0 (30.7, 35.4)
		All 4 strike (n=78)	30.5 (28.4, 32.7)
29--36	2	TBI (n=60)	36.0 (33.0, 39.2)
	4	TBI (n=26)	69.1 (65.2, 73.4)
43--50	2	TBI (n=136)	43.8 (42.1, 45.6)
	4	TBI (n=24)	74.7 (69.8, 80.0)

In young (1–8 day) flies, mortality among the four condition involving only HIT are not all identical ($F_{3,74} = 6.47$, $p = 0.001$) and instead separate as PostISO and the aggregate of the other three with no appreciable loss of information ($F_{2,74} = 0.530$, $p = 0.591$). The forced combination of all four groups results in overall mortality of 30.5% (95% CI: 28.4–32.7%) and is only used for Figure 1 of the original paper; separate comparisons involving each of the four conditions compared against HIT use condition-specific estimates (below) and in other figures within the original paper.

Young flies exposed to HIT and one of the four anesthetic conditions had mortality rates that segregated according to post-exposure and pre-exposure conditions with no real loss of information ($F_{2,74} = 0.458$, $p = 0.634$). Under post-exposure conditions the mortality was 34.5% , while under pre-exposure conditions it was just over 10 percentage points lower (24.1%). This leads to a 46% (95% CI: 21–77%, $p < 0.001$) increase in mortality for the postISO HIT condition relative to just HIT alone. For pre-exposure conditions (preISO, preSEVO), there is a 27% reduction in mortality ($p < 0.001$). Mortality for postSEVO remained relatively constant for the two treatments (34.5% vs 33.0%; $p = 0.527$). These conclusions are summarized in Table 10.

Table 10A. Percent mortality for flies exposed to four strikes under different treatments and conditions. No restrictions or constraints (all 8 values kept separate) with fold changes of GAH/H shown and p-values testing whether actual fold change differs from 1.

Condition	Age 1--8 days for both treatments: GAH	H	Fold (GAH/H)	p-val
postISO	35.5 (31.4, 40.1)	23.5 (20.0, 27.7)	1.51 (1.23, 1.85)	<0.001
postSEVO	30.8 (23.9, 39.8)	30.4 (23.5, 39.4)	1.01 (0.70, 1.46)	0.941
preISO	24.3 (21.4, 27.6)	34.0 (30.7, 37.6)	0.72 (0.61, 0.84)	<0.001
preSEVO	23.8 (20.1, 28.1)	32.1 (28.0, 36.8)	0.74 (0.60, 0.92)	<0.007

Two equality constraints per treatment
GAH : postISO=postSEVO , preISO=preSEVO ; F(2,74)=0.458, p=0.634
H : postSEVO=preISO=preSEVO ; F(2,74)=0.530, p=0.591

Table 10B. Percent mortality under imposition of constraints

Condition	Age 1--8 days for both treatments GAH	H	Fold (GAH/H)	p-val
postISO	34.5 (30.6, 38.9)	23.5 (20.3, 27.4)	1.46 (1.21, 1.77)	<0.001
postSEVO	34.5 (30.6, 38.9)	33.0 (30.7, 35.4)	1.05 (0.91, 1.20)	0.527
preISO	24.1 (21.6, 26.9)	33.0 (30.7, 35.4)	0.73 (0.64, 0.83)	<0.001
preSEVO	24.1 (21.6, 26.9)	33.0 (30.7, 35.4)	0.73 (0.64, 0.83)	<0.001

Table 4. Estimated mortality rate (deaths per 1000 flies exposed; age 1–8 days) for all treatments and conditions. Fold changes (GAH/H) are given with p-values testing whether actual fold change deviates from 1.

	C		GA		GAH		H		Fold-change (GAH/H)		
	Est.	(95% CI)	Est.	(95% CI)	Est.	(95% CI)	Est.	(95% CI)	Est.	(95% CI)	p-val
PostHYPR	0.618	(0.161, 2.38)	4.33	(2.57, 7.30)	157	(120, 206)	137	(126, 149)	1.15	(0.87, 1.53)	0.334
PostHYPRiso	0.618	(0.161, 2.38)	4.33	(2.57, 7.30)	181	(143, 229)	137	(126, 149)	1.32	(1.03, 1.70)	0.029
PostHYPRsevo	0.618	(0.161, 2.38)	4.33	(2.57, 7.30)	144	(110, 188)	137	(126, 149)	1.05	(0.79, 1.39)	0.723
PostISO	0.618	(0.161, 2.38)	4.33	(2.57, 7.30)	177	(141, 222)	137	(126, 149)	1.30	(1.02, 1.65)	0.035
PostSEVO	0.618	(0.161, 2.38)	4.33	(2.57, 7.30)	167	(126, 221)	137	(126, 149)	1.22	(0.91, 1.64)	0.187
PreHYPR	0.618	(0.161, 2.38)	4.33	(2.57, 7.30)	110	(80, 152)	137	(126, 149)	0.80	(0.58, 1.12)	0.201
PreHYPRiso	0.618	(0.161, 2.38)	4.33	(2.57, 7.30)	69	(47, 101)	137	(126, 149)	0.50	(0.34, 0.74)	0.001
PreHYPRsevo	0.618	(0.161, 2.38)	4.33	(2.57, 7.30)	80	(56, 114)	137	(126, 149)	0.59	(0.41, 0.84)	0.004
PreISO	0.618	(0.161, 2.38)	4.33	(2.57, 7.30)	75	(57, 99)	137	(126, 149)	0.55	(0.42, 0.73)	<0.001
PreSEVO	0.618	(0.161, 2.38)	4.33	(2.57, 7.30)	67	(45, 98)	137	(126, 149)	0.49	(0.33, 0.72)	<0.001

Table 6. Estimated mortality rate (deaths per 100 flies exposed; age 43–50 days) for all treatments and conditions. Fold changes (GAH/H) are given with p-values testing whether actual fold change deviates from 1.

	C		GA		GAH		H		Fold-change (GAH/H)		
	Est.	(95% CI)	Est.	(95% CI)	Est.	(95% CI)	Est.	(95% CI)	Est.	(95% CI)	p-val
PostHYPR	1.46	(0.85, 2.49)	8.30	(5.30, 13.02)	59.0	(51.9, 67.1)	43.8	(42.1, 45.6)	1.35	(1.19, 1.52)	<0.001
PostHYPRiso	0.33	(0.11, 0.96)	15.68	(11.52, 21.34)	61.7	(54.8, 69.3)	43.8	(42.1, 45.6)	1.41	(1.26, 1.57)	<0.001
PostHYPRsevo	1.46	(0.85, 2.49)	3.18	(1.58, 6.42)	51.4	(44.7, 59.1)	43.8	(42.1, 45.6)	1.17	(1.03, 1.34)	0.017
PostISO	1.46	(0.85, 2.49)	4.05	(2.20, 7.47)	49.9	(43.3, 57.4)	43.8	(42.1, 45.6)	1.14	(1.00, 1.30)	0.054
PostSEVO	1.46	(0.85, 2.49)	3.54	(1.62, 7.74)	54.7	(47.1, 63.5)	43.8	(42.1, 45.6)	1.25	(1.09, 1.43)	0.002
PreHYPR	0.33	(0.11, 0.96)	4.27	(2.13, 8.58)	50.2	(43.2, 58.2)	43.8	(42.1, 45.6)	1.14	(1.00, 1.31)	0.054
PreHYPRiso	0.33	(0.11, 0.96)	7.69	(4.80, 12.34)	54.0	(47.6, 61.4)	43.8	(42.1, 45.6)	1.23	(1.09, 1.39)	0.001
PreHYPRsevo	0.33	(0.11, 0.96)	2.72	(1.24, 5.97)	42.7	(36.3, 50.1)	43.8	(42.1, 45.6)	0.97	(0.84, 1.13)	0.715
PreISO	5.66	(3.86, 8.30)	9.92	(6.47, 15.21)	50.4	(42.8, 59.4)	43.8	(42.1, 45.6)	1.15	(0.99, 1.34)	0.069
PreSEVO	5.66	(3.86, 8.30)	4.79	(2.66, 8.63)	43.3	(36.6, 51.4)	43.8	(42.1, 45.6)	0.99	(0.85, 1.15)	0.885