Supplementary Material 1

Doses (mg/Kg) of drugs used in male and female rats submitted to different anesthetic associations (X: Xylazine, K: Ketamine, A: Acepromazine, Me: Methadone, Mo: Morphine, T: Tramadol).

	Anesthetic protocols (mg/Kg)			
Sex	XKA	XKMe	XKMo	XKT
Male rats	7,5 + 60 + 2	5 + 60 + 5	7 + 60 + 1	7,5 + 65 + 5
Female rats	5 + 60 + 1	5 + 60 + 5	5 + 60 + 1,5	5 + 60 + 5

Supplementary Material 2

Oxygen saturation (SO2)

The best fit and well supported model for explaining the variation of SO2 in male and female rats was the time only model (AICcw = 1.0, Table S1). Prior to oxygen administration saturation levels were below normal ranges (<90%) in both male and females. After the administration of oxygen at the minute 7, an immediate recovery of the saturation occurred and in most of the cases exceeded 95% (Figure S1). The XKMo protocol in females had a lower SO2 after 40 minutes but within an adequate percentage (Figure S1B).

Table S1 Candidate set of Linear Mixed Effects Models used to test the influence of the anesthesia on the oxygen saturation (SO2) evaluated in rats submitted to different anesthetic associations. The prioritization of the models was based on the Akaike Information Criterion (AIC) adjusted for small samples (AICc) and separated according to sex.

	Male rats				Female rats			
Model	k[a]	AICc	Δ AICc[b]	AICcw[c]	k[a]	AICc	Δ AICc[b]	AICcw[c]
SO2								
Time	9	1165,81	0,00	1,00	8	914,28	0,00	1,00
Time x Protocol	30	1189,49	23,68	0,00	26	949,23	34,96	0,00
Null model	3	1296,49	130,68	0,00	3	1011,79	97,51	0,00
Protocol	6	1299,08	133,27	0,00	6	1015,41	101,13	0,00

a Number of estimated parameters in the model
b Difference in value between AICc of the current model versus the best-approximating model (AICc min)
c Akaike weight. Probability that the current model (i) is the best-approximating model among those considered.

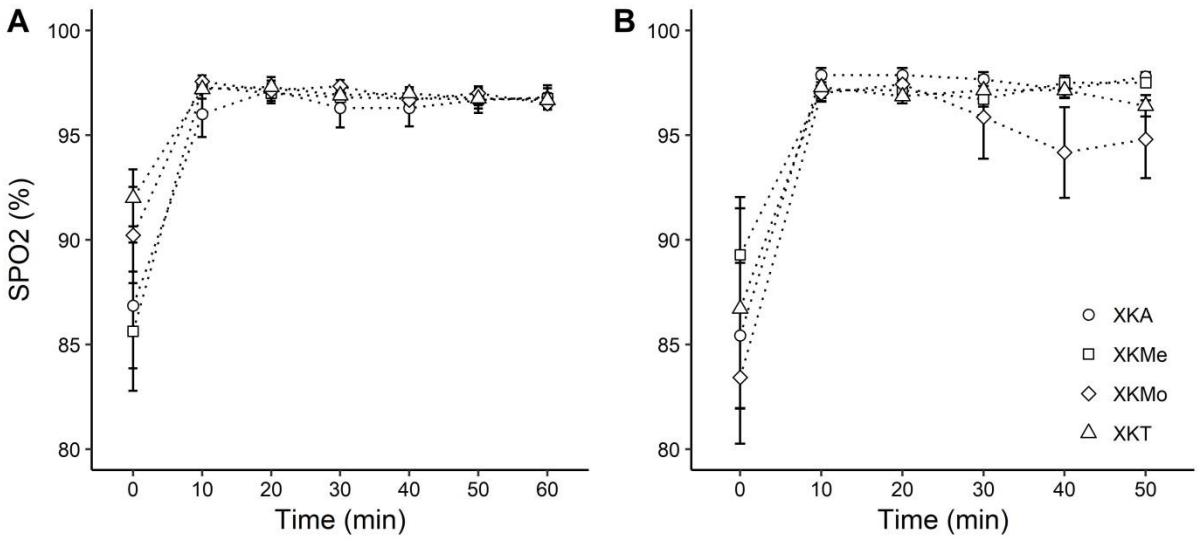

Fig S1 Variation of oxygen saturation (%) in male (A) and female (B) rats submitted to different anesthetic associations (X: Xylazine, K: Ketamine, A: Acepromazine, Me: Methadone, Mo: Morphine, T: Tramadol). The bars represent the standard error of the mean.

Discussion of these results

The decrease in oxygen saturation (SO2) coincides exactly with the respiratory depression at minute 10, falling rapidly to levels below 90%. Values lower than 90% of SO2 are a cause of concern because they are correlated with an oxygen partial pressure (pO2) of 60 mmHg (minimum value corresponding to the reference range), an additional decrease in saturation can result in a large drop in pO2 that can lead to a hypoxemia.[1] In several animal models, hypoxemia has been shown to cause harmful effects in almost all organs at the cellular level, leading to changes such as acute heart

failure, pulmonary hypertension, acute renal failure, and damage to neuronal tissue with decreased cognitive function.[2-5]

Immediately after oxygen administration, the immediate improvement in the saturation indicates that the oxygen concentration in blood increase, confirming that this practice is necessary during procedures using injectable anesthesia, especially in those that adopt the same associations of this study. Similarly, Fornari et al. 2012 administered oxygen during a surgical procedure performed with the injectable anesthetic association of ketamine and dexmedetomidine in rats, and demonstrated that the oxygenated animals showed an increase in the survival rate and a significantly lower post-surgical weight loss than those who did not receive oxygen, confirming the beneficial effect of this practice.

References

1. Flecknell P. *Laboratory Animal Anesthesia*. 3rd ed. Newcastle: Elsevier Inc, 2009, p.300.
2. Brezis M and Rosen S. Hypoxia of the renal medulla–its implications for disease. *N. Engl. J. Med* 1995; 332: 647–55.
3. Cross CE, Rieben PA, Barron CI et al. Effects of arterial hypoxia on the heart and circulation: an integrative study. *Am. J. Physiol* 1963; 205: 963–970.
4. Gozal D, Daniel JM, Dohanich GP. Behavioral and anatomical correlates of chronic episodic hypoxia during sleep in the rat. *J Neurosci* 2001; 21: 2442–2450.
5. Zielinski J. Effects of intermittent hypoxia on pulmonary haemodynamics: animal models versus studies in humans. *Europ Respir J* 2005; 25:173–180.

Supplementary Material 3

Candidate set of Linear Mixed Effects Models used to test the influence of the anesthesia on the different physiological parameters evaluated in rats submitted to different anesthetic associations. The prioritization of the models was based on the Akaike Information Criterion (AIC) adjusted for small samples (AICc) and separated according to sex.

	Male rats				Female rats			
Model	k[a]	AICc	Δ AICc[b]	AICcw[c]	k[a]	AICc	Δ AICc[b]	AICcw[c]
Temperature								
Time	9	424.54	0.00	1.00	8	298.93	0.00	1.00
Time x Protocol	30	456.16	31.62	0.00	3	316.98	18.05	0.00
Null model	3	482.55	58.01	0.00	6	318.92	19.99	0.00
Protocol	6	486.07	61.53	0.00	26	322.92	23.99	0.00
Heart Rate								
Time	9	2013.79	0.00	1.00	8	1481.91	0.00	1.00
Time x Protocol	30	2043.47	29.68	0.00	26	1492.96	11.05	0.00
Null model	3	2138.74	124.95	0.00	6	1517.84	35.93	0.00
Protocol	6	2143.54	129.75	0.00	3	1532.16	50.26	0.00
Respiratory Rate								
Time	9	2063.60	0.00	1.00	8	1601.22	0.00	0.96
Time x Protocol	30	2075.94	12.34	0.00	26	1607.77	6.55	0.04
Null Model	3	2184.85	121.25	0.00	6	1633.58	32.36	0.00
Protocol	6	2190.39	126.78	0.00	3	1635.71	34.49	0.00

a Number of estimated parameters in the model
b Difference in value between AICc of the current model versus the best-approximating model (AICcmin)
c Akaike weight. Probability that the current model (i) is the best-approximating model among those considered.

Supplementary Material 4

Pairwise comparisons of temperatures across time derived from the best fit model. Mean difference (Estimate), standard error (SE), and 95% confidence intervals (95% CIs). Only the comparisons when 95% CIs do not overlap 0 are shown.

Temperature							
Male rats				Female rats			
Comparison (time)	Estimate	SE	95% CI	Comparison (time)	Estimate	SE	95% CI
0 – 10	0,61	0,12	0.38 – 0.85	0 –10	0.53	0.13	0.27 – 0.79
0 – 20	0.41	0.12	0.18 – 0.65	10 – 20	-0.38	0.13	-0.63 – -012
0 – 50	-0.35	0.14	-0.62 – -0.09	10 – 30	-0.54	0.13	-0.80 – -0.28
0 – 60	-0.34	0.15	-0.64 – -0.04	10 – 40	-0.61	0.13	-0.87 – -0.34
10 – 30	-0.49	0.12	-0..73 – -0.26	10 – 50	-0.67	0.14	-0.95 – -0.38
10 – 40	-0.73	0.12	-0.98 – -0.49				
10 – 50	-0.97	0.14	-1..23 – -0.70				
10 – 60	-0.95	0.15	-1.25 – 0.65				
20 – 30	-0.29	0.12	-0..53 – -0.06				
20 – 40	-0.53	0.12	-0..78 – -0.29				
20 – 50	-0.77	0.14	-1..03 – -0.50				
20 – 60	-0.75	0.15	-1.05 – -0.45				
30 – 50	-0.47	0.14	-0.74 – -0.20				
30 – 60	-0.46	0.15	-0.76 – -0.16				

Supplementary Material 5

Pairwise comparisons of heart rates across time derived from the best fit model. Mean difference (Estimate), standard error (SE), and 95% confidence intervals (95% CI). Only the comparisons when 95%CI do not overlap 0 are shown.

Heart rate							
Male rats				Female rats			
Comparison	Estimate	SE	95% CI	Comparison	Estimate	SE	95% CI
0 – 10	42.79	4.28	34.35 - 51.24	0 - 10	42.61	5.53	31.67 – 61.74
0 – 20	48.11	4.28	39.66 - 56.55	0 - 20	41.68	5.53	30.74 – 47.17
0 – 30	41.24	4.28	32.79 – 49.68	0 – 30	41.35	5.59	30.28 – -15.96
0 – 40	28.95	4.38	20.31 – 37.60	0 – 40	33.00	5.72	21.68 – -35.29
0 – 50	11.67	4.88	2.04 – 21.29	0 – 50	28.12	6.04	16.17 – -36.47
10 – 40	-13.84	4.39	22.5 – 51.7	10 – 50	-14.49	6.04	-26.44 – -20.72
10 – 50	-31.13	4.89	–40.76 – -21.49	20 – 50	-13.56	6.04	-25.51 – -21.90
10 – 60	-40.28	5.48	–51.09 – -29.48	30 – 50	-13.23	6.09	-25.27 –-–2.16
20 – 40	-19.16	4.39	–27.82 – -10.49				
20 – 50	-36.44	4.89	–46.08 – -26.81				
20 – 60	-45.60	5.48	–56.41 – -34.80				
30 – 40	-12.28	4.39	–20.94 – -3.62				
30 – 50	-29.57	4.89	–39.21 – -19.93				
30 – 60	-38.73	5.48	–49.53 – -27.92				
40 – 50	-17.29	4.95	–27.04 – -7.53				
40 – 60	-26.45	5.53	–37.36 – -15.53				

Supplementary Material 6

Pairwise comparisons of respiratory rates across time derived from the best fit model. Mean difference (Estimate). Standard Error (SE), and 95% Confidence Intervals (95% CIs). Only the comparisons when 95% CIs do not overlap 0 are shown.

Respiratory rate							
Male rats				Female rats			
Comparison	Estimate	SE	95% CIs	Comparison	Estimate	SE	95% CIs
0 – 10	47.52	5.28	37.11 – 57.93	0 – 10	43.71	9.11	25.69 – 61.74
0 – 20	47.30	5.28	36.88 – 57.71	0 – 20	29.14	9.11	11.12 – 47.17
0 – 30	40.30	5.28	29.88 – 50.71	10 – 30	-34.18	9.21	-52.40 – -15.96
0 – 40	30.67	5.41	19.99 – 41.35	10 – 40	-53.93	9.42	-72.56 – -35.29
0 – 60	-15.03	6.60	-28.05 – -2.00	10 – 50	-56.12	9.94	-75.78 – -36.47
10 – 40	-16.85	5.44	-27.58 – -6.11	20 – 40	-39.35	9.42	-57.99 – -20.72
10 – 50	-35.76	5.93	-47.45 – -24.06	20 – 50	-41.55	9.94	-61.21 – -21.90
10 – 60	-62.55	6.63	-75.62 – -49.47	30 – 50	-21.94	10.00	-41.73 – -2.16
20 – 40	-16.63	5.44	-27.36 – -5.89				
20 – 50	-35.54	5.93	-47.23 – -23.84				
20 – 60	-62.32	6.63	-75.39 – -49.25				
30 – 50	-28.54	5.93	-40.23 – -2.25				
30 – 60	-55.32	6.63	-68.39 – -7.03				
40 – 50	-18.91	6.02	-30.78 – -32.46				
40 – 60	-45.70	6.71	-58.93 – -12.4				
50 – 60	-26.79	7.02	-40.64 – -12.94				

Supplementary Material 7

Candidate set of Linear Models used to test the influence of the anesthesia on the anesthetic times evaluated in rats submitted to different anesthetic associations. The prioritization of the models was based on the Akaike Information Criterion (AIC) adjusted for small samples (AICc) and separated according to sex.

	Male rats				Female rats			
Model	k[a]	AICc	Δ AICc[b]	AICcw[c]	k[a]	AICc	Δ AICc[b]	AICcw[c]
Induction								
Null model	2	41.55	0.00	0.92	2	42.66	0.00	0.94
Protocol	5	46.42	4.87	0.08	5	48.05	5.39	0.06
Non–surgical anestesia								
Protocol	5	308.12	0.00	1.00	5	58.83	0.00	1.00
Null model	2	323.46	15.34	0.00	2	76.63	17.79	0.00
Surgical anestesia								
Null model	2	93.84	0.00	0.83	2	122.41	0.00	0.94
Protocol	3	96.97	3.13	0.17	4	127.76	5.35	0.06
Recovery								
Protocol	5	299.66	0.00	0.90	5	267.25	2.07	0.26
Null model	2	303.95	4.29	0.10	2	265.19	0.00	0.74
Total duration								
Null model	2	48.01	0.00	0.60	2	280.83	0.00	0.86
Protocol	5	48.83	0.82	0.40	5	284.49	3.67	0.14

a Number of estimated parameters in the model
b Difference in value between AICc of the current model versus the best–approximating model (AICcmin)
c Akaike weight. Probability that the current model (i) is the best–approximating model among those considered.

Supplementary Material 8

Cohen's d pairwise comparisons of the anesthesia times in male and female rats submitted to different anesthetic associations (X: Xilazine, K: Ketamine, A: Acepromazin, Me: Methadone, Mo: Morphine, T: Tramadol). Cohen's d estimates were derived from the best fit model. Values in parenthesis represent 95% CIs of Cohen's d estimates. Italicized values indicate a Cohen's d estimate with 95% CIs non–overlapping zero. Estimates with 95% CIs overlapping zero suggests a non–significant difference in the respective pairwise comparison.

	Non–surgical anesthesia		Recovery		Total duration	
Comparison	Male rats	Female rats	Male rats	Female rats	Male rats	Female rats
XKA – XKMe	-0.90 (-2.10 – 0.30) [e]	0.10 (-1.00 – 1.50) [b]	1.8 (-0.40 – 3.10) [f]	1.8 (-0.20 – 2.50) [f]	0.8 (-0.40 – 2.00) [e]	0.0 (-1.30 – 1.20) [a]
XKA – XKMo	-2.20 (-3.70 – –0.70) [g]	-1.80 (-3.40 – -0.30) [f]	0.5 (-0.70 – 1.80) [d]	0.3 (-1.00 – 1.50) [c]	0.1 (-1.10 – 1.30) [b]	-0.3 (-1.60 – 0.90) [c]
XKA – XKT	-5.00 (-7.40 – –2.70) [g]	-1.7 (-3.19 – -0.20) [f]	1.4 (0.10 – 2.80) [f]	0.5 (-0.80 – 1.80) [d]	1.1 (-0.20 – 2.30) [e]	0.7 (-0.60 – 2.00) [d]
XKMe – XKMo	-0.90 (-2.00 – 0.20) [e]	-2.1 (-3.70 – -0.50) [g]	–0.9 (-2.00 – 0.10) [e]	-1.0 (-2.40 – 0.30) [e]	-0.8 (-1.80 – 0.33) [e]	-0.4 (-1.60 – 0.90) [c]
XKT – XKMe	-1.30 (-2.40 – -0.20) [f]	-2.1 (-3.70 – -0.40) [g]	–0.1 (-1.10 – 0.80) [b]	-1.6 (-3.10 – -0.10) [f]	0.0 (-0.90 – 1.00) [a]	1.2 (-0.10 – 2.70) [f]
XKMo – XKT	0.10 (-0.90 – 1.20) [b]	0.5 (-0.80 – 1.80) [d]	0.7 (-0.30 – 1.80) [d]	0.2 (-1.02 – 1.50) [c]	1.0 (-0.10 – 2.10) [e]	1.3 (-0.20 – 2.50) [f]

a: negligible, b: very small., c:small, d: medium, e: large, f: very large, g: huge

Supplementary Material 9

Candidate set of Generalized Linear Mixed Models used to evaluate the influence of the anesthesia on the number of ultrasound vocalizations evaluated in rats submitted to different anesthetic associations. The prioritization of the models was based on the Akaike Information Criterion (AIC) adjusted for small samples (AICc) and separated according to sex.

	Male rats				Female rats			
Model	k[a]	AICc	Δ AICc[b]	AICcw[c]	k[a]	AICc	Δ AICc[b]	AICcw[c]
Number of events								
Time x Protocol	17	716.20	0.00	1.00	17	504.63	0.00	1.00
Time	5	852.81	136.60	0.00	5	570.69	66.07	0.00
Protocol	5	1002.85	286.63	0.00	5	814.74	310.11	0.00
Null model	2	1007.90	291.69	0.00	2	816.80	312.17	0.00

a Number of estimated parameters in the model
b Difference in value between AICc of the current model versus the best–approximating model (AICcmin)
c Akaike weight. Probability that the current model (i) is the best–approximating model among those considered.