Supplementary Material

Medullary norepinephrine neurons modulate local oxygen concentrations in the bed nucleus of the stria terminalis

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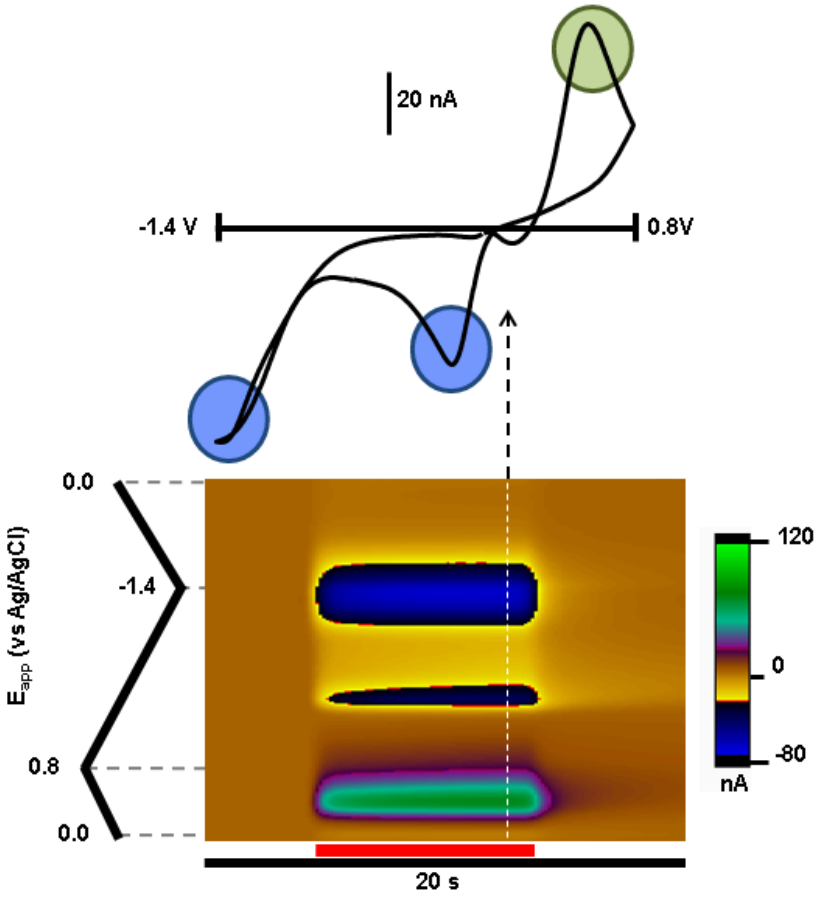
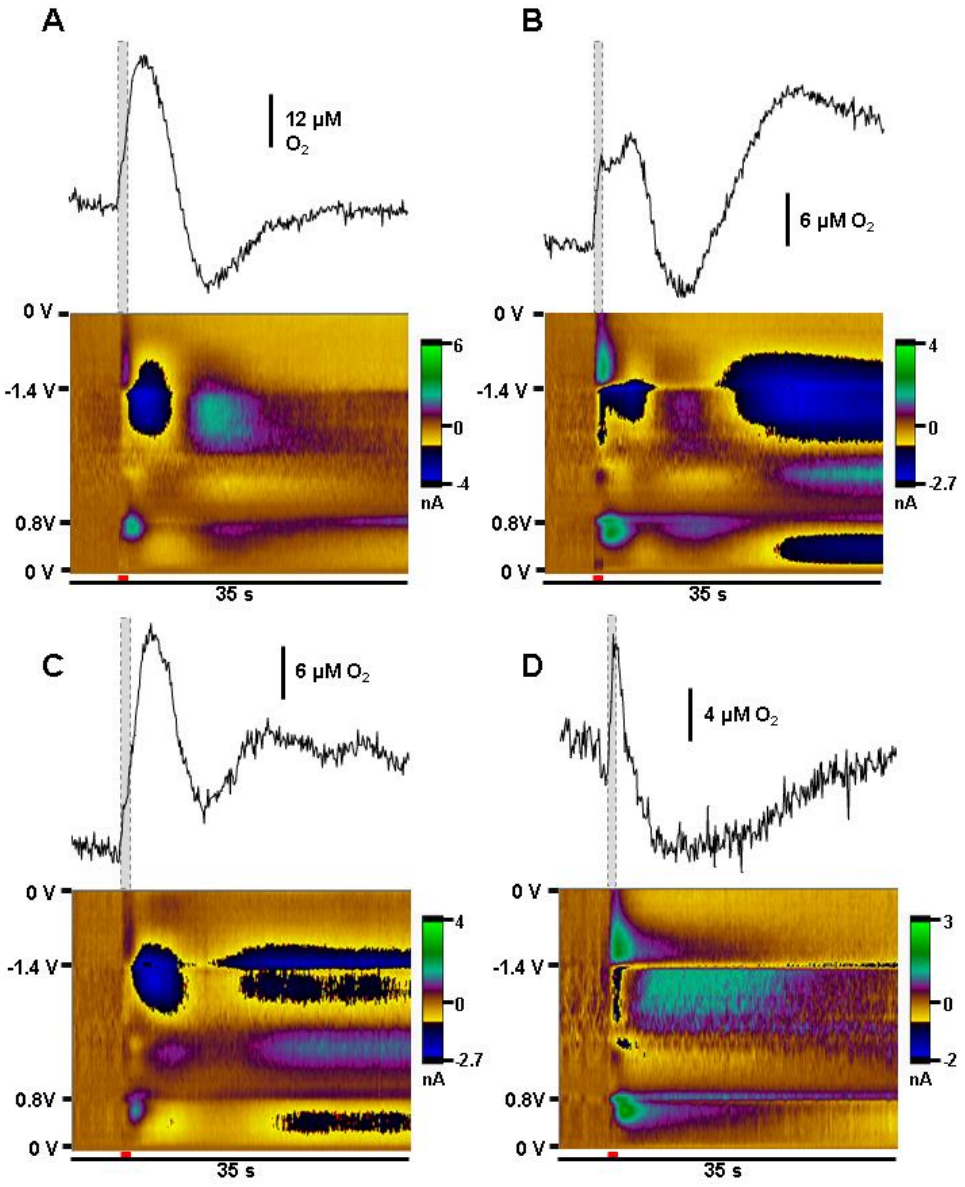
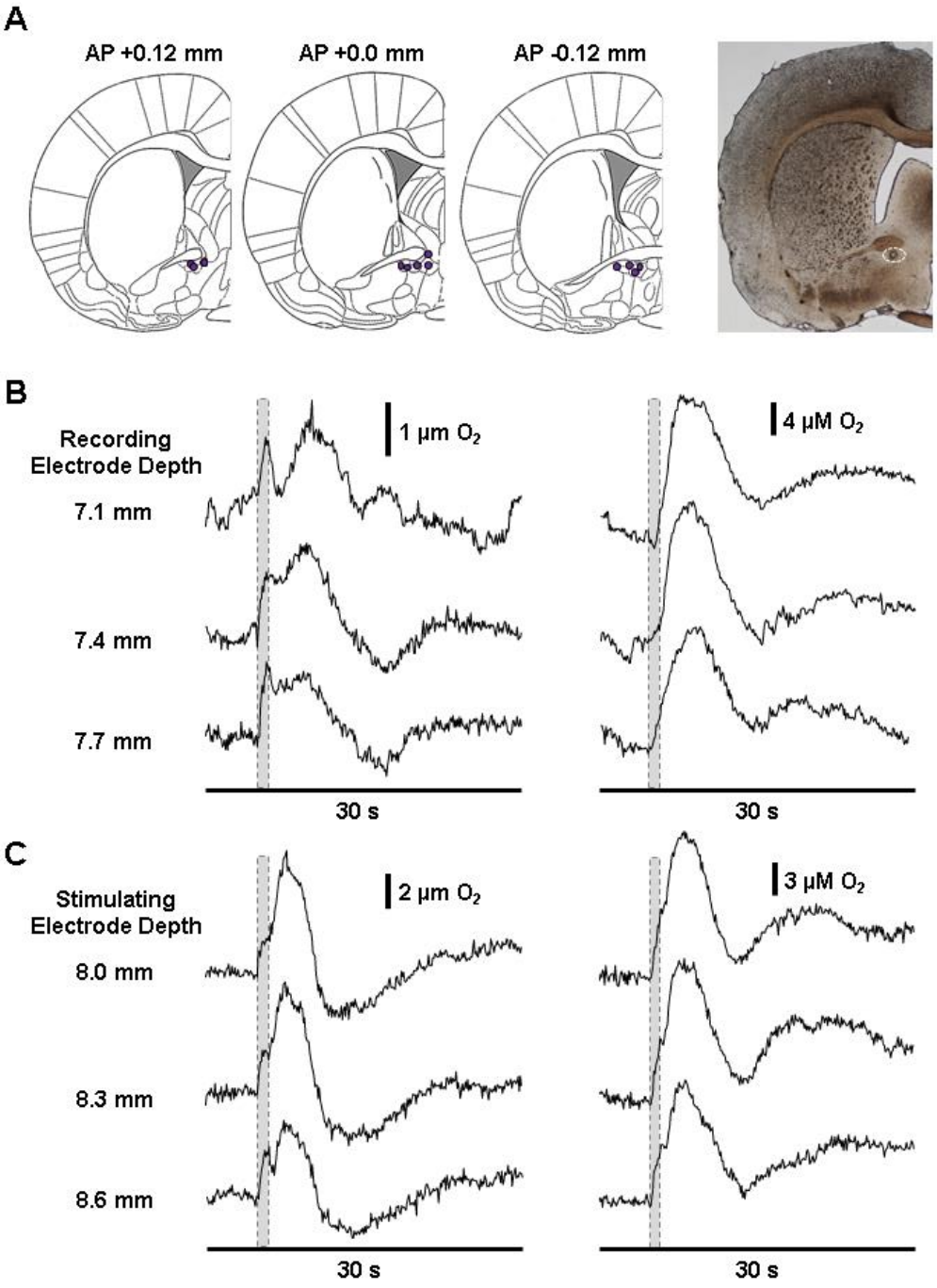


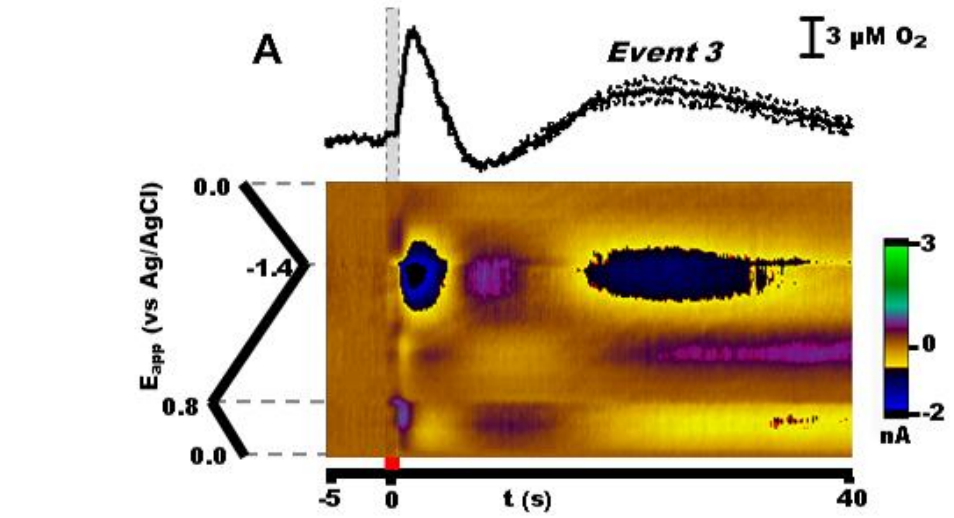
Supplemental Fig 1. Simultaneous detection of 250 µM O_2 and 8 µM norepinephrine in an air-impermeable flow injection system. Redox currents are displayed in a color plot with the time of injection indicated by the red bar. The oxidation of norepinephrine and the reduction of its *o*-quinone produce currents at +0.75 V (anodic scan) and -0.2 V (cathodic scan) respectively. The reduction of molecular O_2 generates currents which peak at the -1.4 V switching potential. These features are readily apparent in a cyclic voltammogram exacted from the time of sample injection.

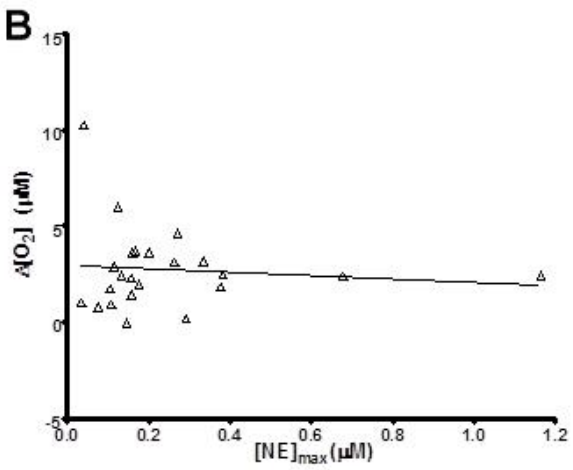


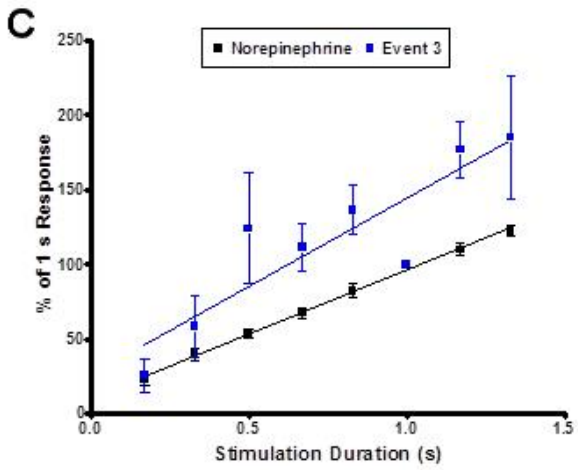
Supplemental Fig 2. Variability of the O_2 response to electrical stimulation of the ventral noradrenergic bundle. Stimulation times are indicated by the red and gray-dashed bars. Approximately 65% of recording locations exhibited an increase in O_2 followed by a transient decrease below baseline (A). In many of these locations the decrease in O_2 was followed by a second prolonged increase in extracellular levels (B). In other recording locations (~ 30%) O_2 concentrations biphasically increased with the electrical stimulation (C). In a small number of animals (~ 5%) the stimulation resulted in a monophasic O_2 decrease (D). Only responses that resembled (A) and (B) were characterized for the purposes of this study.

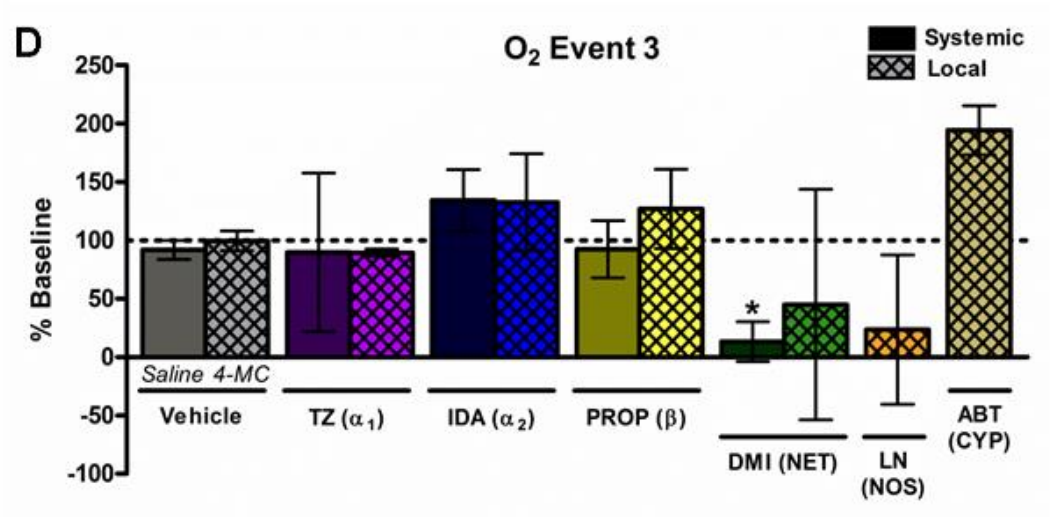


Supplemental Fig 3. Effect of electrode placements on the recorded O_2 response. (A) Representative histological verification of the recording sites in the ventral bed nucleus of the stria terminalis (vBNST). Coronal diagrams are adapted from the atlas of Paxinos and Watson. Placement of the carbon-fiber microelectrode was determined by electrolytic lesion (left). (B - C) The O_2 response did not vary with the depth of the recording and stimulating electrodes. Example dorsal-ventral profiles are shown for the increase-decrease O_2 response type (B-C, left) and for animals exhibiting a biphasic O_2 increase (B-C, right).

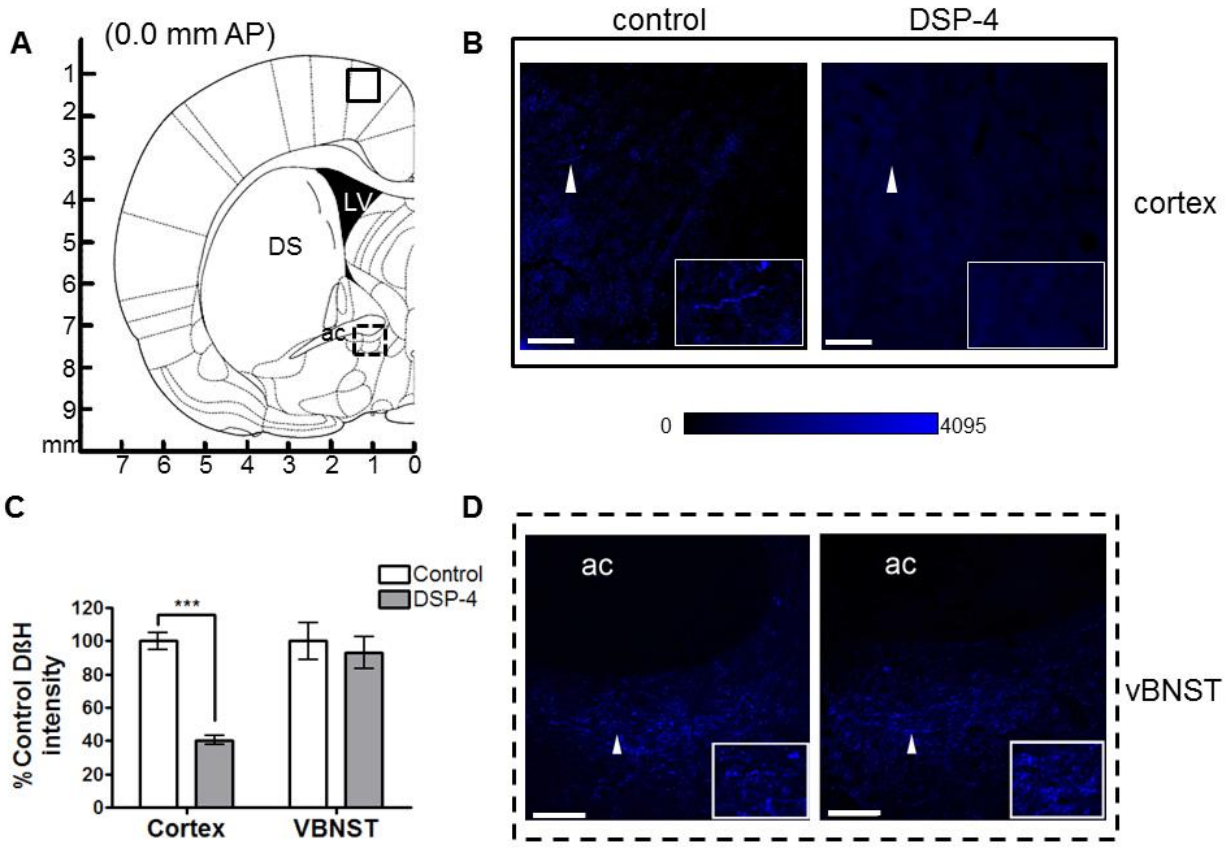

A
Event 3
3 µM O2
0.0
-1.4
0.8
0.0
Eapp (vs Ag/AgCl)
3
0
-2
nA
-5
0
t (s)
40


B
Δ[O2] (µM)
[NE]max (µM)

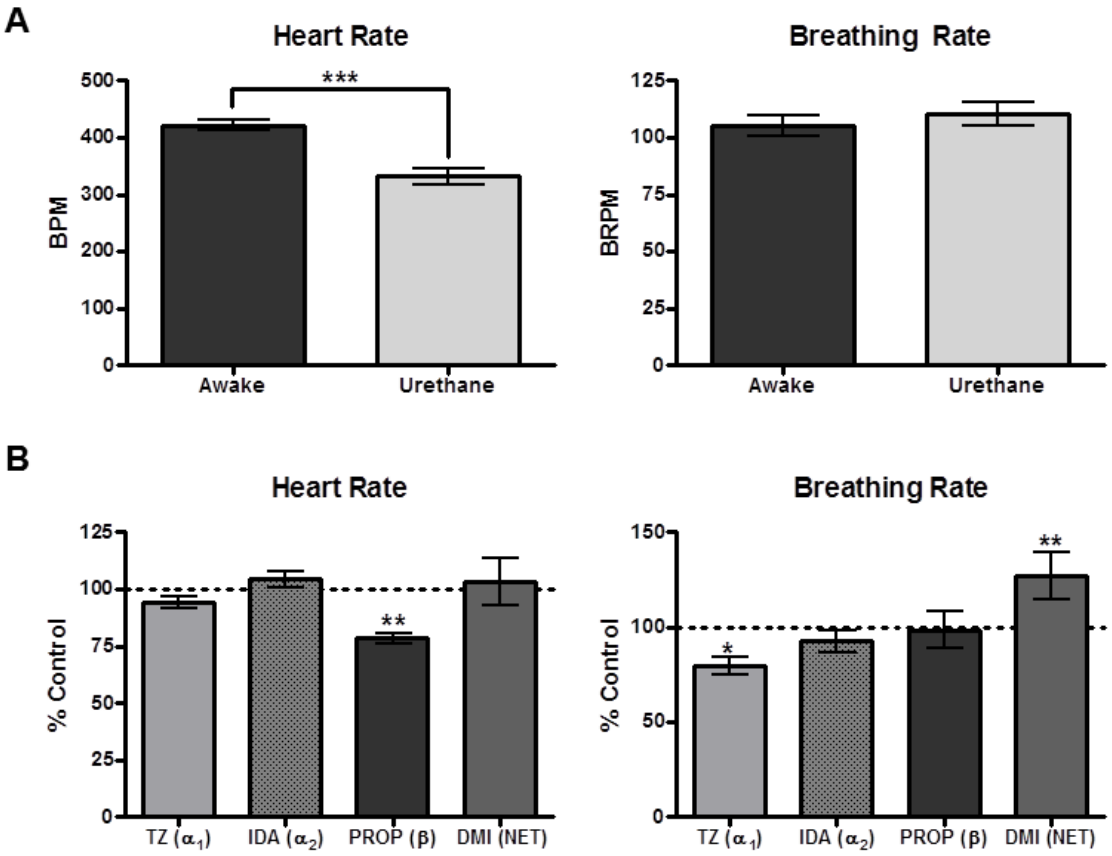

C
Norepinephrine
Event 3
% of 1 s Response
Stimulation Duration (s)


D
O2 Event 3
Systemic
Local
% Baseline
Saline 4-MC
Vehicle
TZ (α1)
IDA (α2)
PROP (β)
DMI (NET)
LN (NOS)
ABT (CYP)

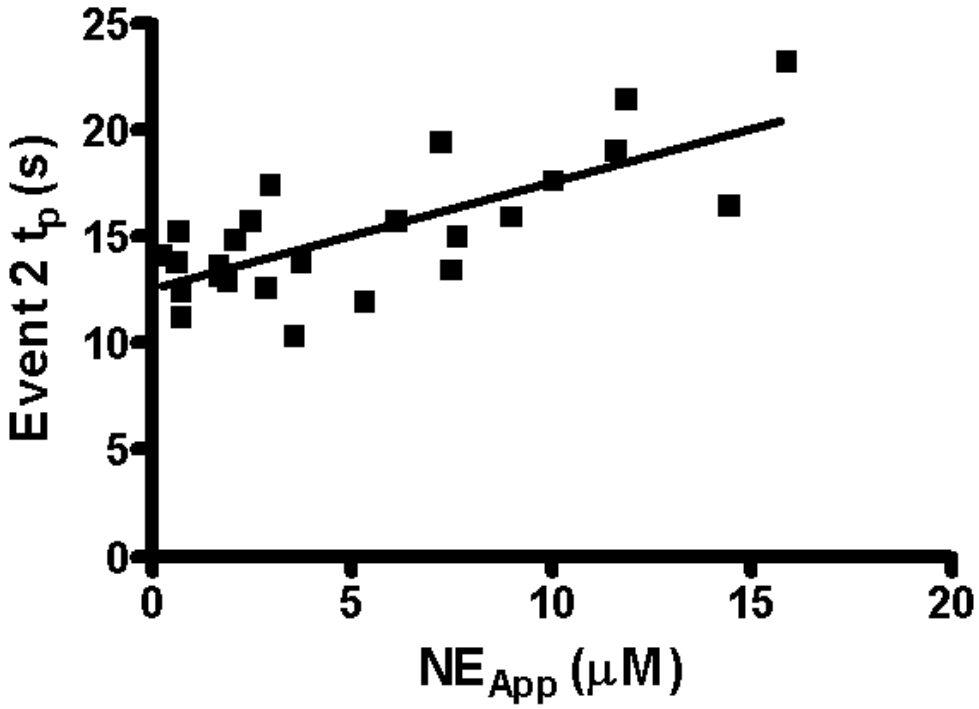
Supplemental Fig 4. Characterization of the second O_2 increase induced by electrical-stimulation of the ventral noradrenergic bundle. (A) The second O_2 increase, denoted as event 3, is observable in an extended time view of the electrically-stimulation response. (B) The magnitude of the event 3 is poorly correlated with the concentration of norepinephrine released by stimulation (n = 23, r^2 = 0.01). (C) Average peak amplitudes for norepinephrine and event 3 as a function of stimulation pulse number (n = 5, 10 to 80 pulses). Each data set is normalized to the 60 pulse response. Event 3 increased linearly within this pulse range (r^2 = 0.31). The slope of its response was not significantly different than that of norepinephrine. (D) Pharmacology of event 3. Systemic drug administration (solid) was performed through *i.p.* injection. Local drug administration (crosshatch) was accomplished through iontophoretic ejection. Saline (n = 21), 4-methylcatechol (4-MC, n = 4), terazosin (TZ, n = 5 systemic, n = 4 local), idazoxan (IDA, n = 5 systemic, n = 4 local), propranolol (PROP, n = 4 systemic, n = 5 local), local desipramine (DMI, n=4), L-NAME (LN, n=4) and ABT (n = 3) did not statistically affect event 3. Systemic desipramine (DMI) significantly decreased the amplitude this event ($P < 0.05$, n = 4). Significance was determined by a one-way ANOVA with a Bonferroni post hoc test comparing drug to its vehicle control (saline or 4-MC).



Supplemental Fig 5. The effect of DSP-4 lesioning on DBH immunoreactivity. (A) Locations in the cortex (solid box) and the ventral bed nucleus of the stria terminalis (vBNST, dashed box) used to obtain the fluorescence data in B - D. Diagram adapted from the atlas of Paxinos and Watson. (C) Identical acquisition parameters were employed to detect changes in DBH immunoreactivity between control and DSP-4 treated animals. Significant changes were noted in cortical but not vBNST sections. Intensity is in arbitrary units, and represents a ratio of Alexa Fluor-488 conjugated goat anti-rabbit fluorescence emission in treated compared to untreated animals. Representative images are shown for cortical (B) and vBNST (D) sections. Scale bar = 100µm, 35 µm for inset images.



Supplemental Fig 6. Cardiorespiratory responses to anesthesia and adrenoceptor antagonists. (A) Effect of 1.5 g/kg urethane on heart and breathing rate. At this dose, there was a significant reduction in heart rate (n=16, P<0.001, two-tailed paired student's t-test), but no change in breathing rate. (B) Heart and breathing rates after i.p. administration of terazosin (TZ), idazoxan (IDA), propranolol (PROP) and desipramine (DMI). Significance determine by a Dunnet's post hoc test following a one-way ANOVA, n=4 for each. *P<0.05, **P<0.01, ***P<0.001. BPM: beats per minute, BRPM: breaths per minute.



Supplemental Fig 7. Effect of norepinephrine concentration applied by iontophoresis (NE_{app}) on the peak time (t_p) for event 2. The minima for event 2 became more delayed at higher norepinephrine concentrations (n =5, slope = 0.50 ± 0.10 s/ μM norepinephrine, r^2 = 0.54).