Science Advances

AAAS

advances.sciencemag.org/cgi/content/full/4/2/eaaq0800/DC1

Supplementary Materials for

Cardiorespiratory interactions previously identified as mammalian are present in the primitive lungfish

Diana A. Monteiro, Edwin W. Taylor, Marina R. Sartori, André L. Cruz, Francisco T. Rantin, Cleo A. C. Leite

Published 21 February 2018, *Sci. Adv.* **4**, eaaq0800 (2018)
DOI: 10.1126/sciadv.aaq0800

This PDF file includes:

Supplementary Materials

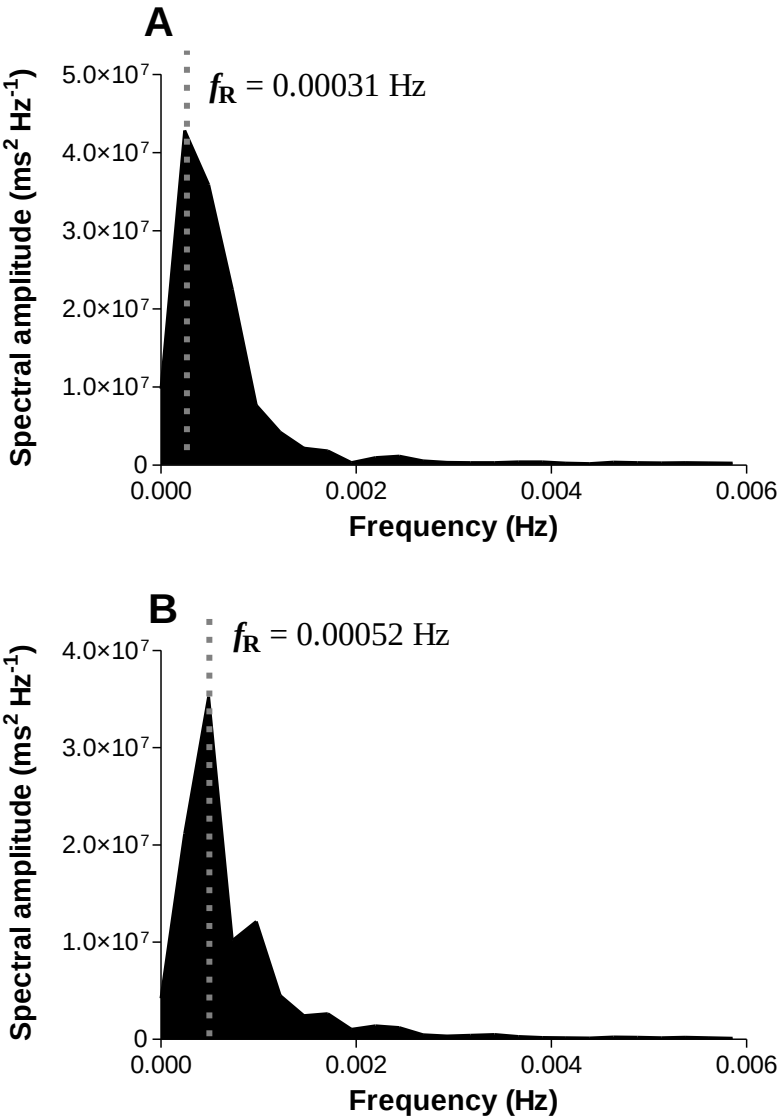

fig. S1. Spectral amplitude density from two lungfish (*L. paradoxa*) with different breathing frequencies. The power spectra for HRV of two lungfish **(A: 196 g; B: 263 g)**, having different regular rates of air breathing (indicated by the dashed line) 24h after their placement in the respirometer. Each fish showed a peak in its power spectrum that corresponded to its individual air breathing frequency (f_R). For reference, the Very Low Frequency (VLF) components distinguished in spectra from resting human subjects are located between 0.003 to 0.04 Hz (Task Force, 1986).

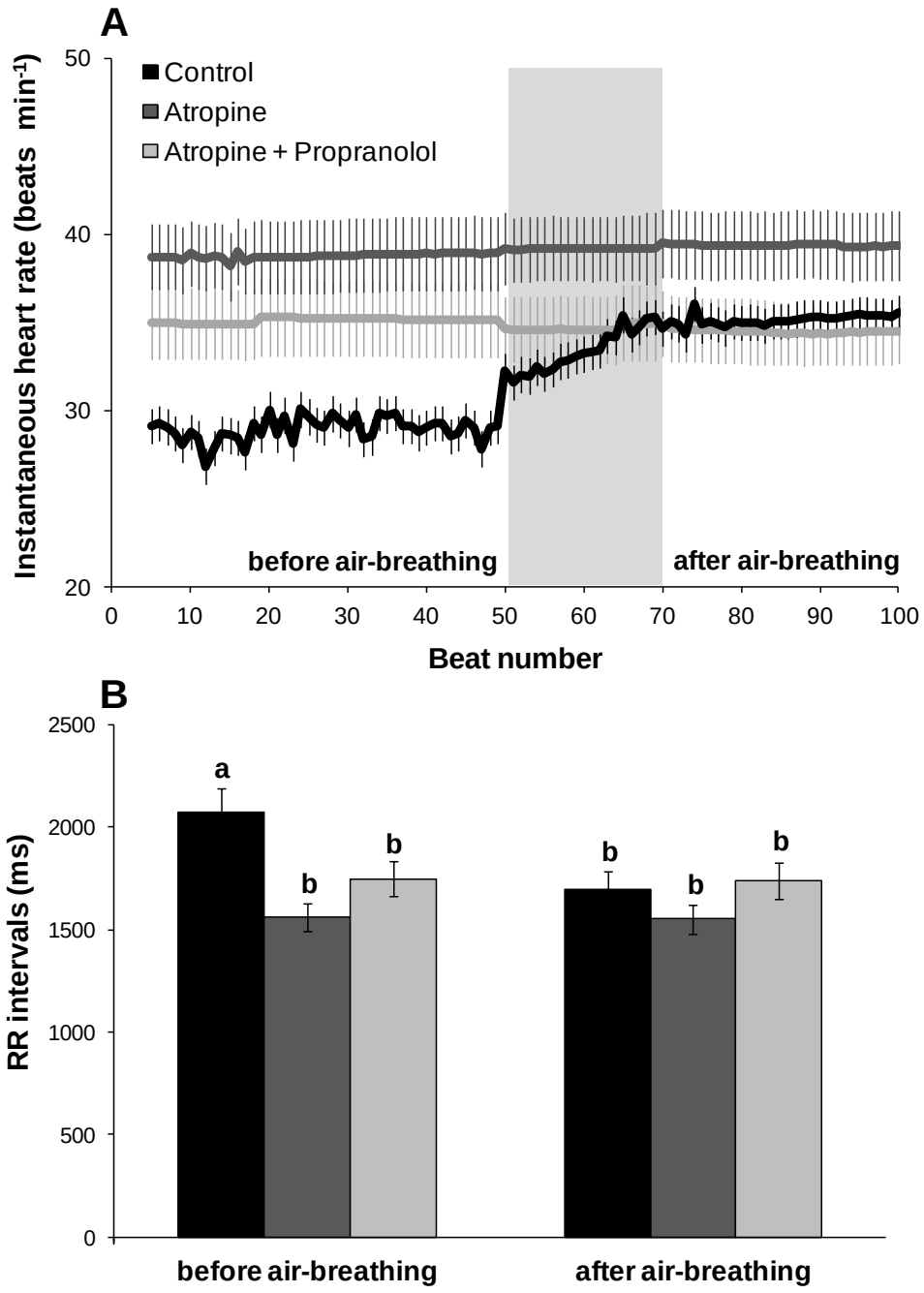

fig. S2. Changes in the heart rate in *L. paradoxa* during an air-breathing event at 25ºC. (**A**) Mean instantaneous heart rate recorded over 100 heart beats around each air breath. Light gray area represents duration of air-breathing cycle; and (**B**) RR*i* intervals under: untreated condition (Control, n = 8); after cholinergic receptor blockade (Atropine, n = 6); after total β-adrenergic and cholinergic receptor blockade (Atropine + Propranolol, n = 6). Black, dark grey and grey colors (top left indication) denote experimental condition for both panels. **A**. Data are plotted as means ± S.E.M. Different letters in **B** denote significantly different mean values (ANOVA Tukey post-hoc $p<0.05$).

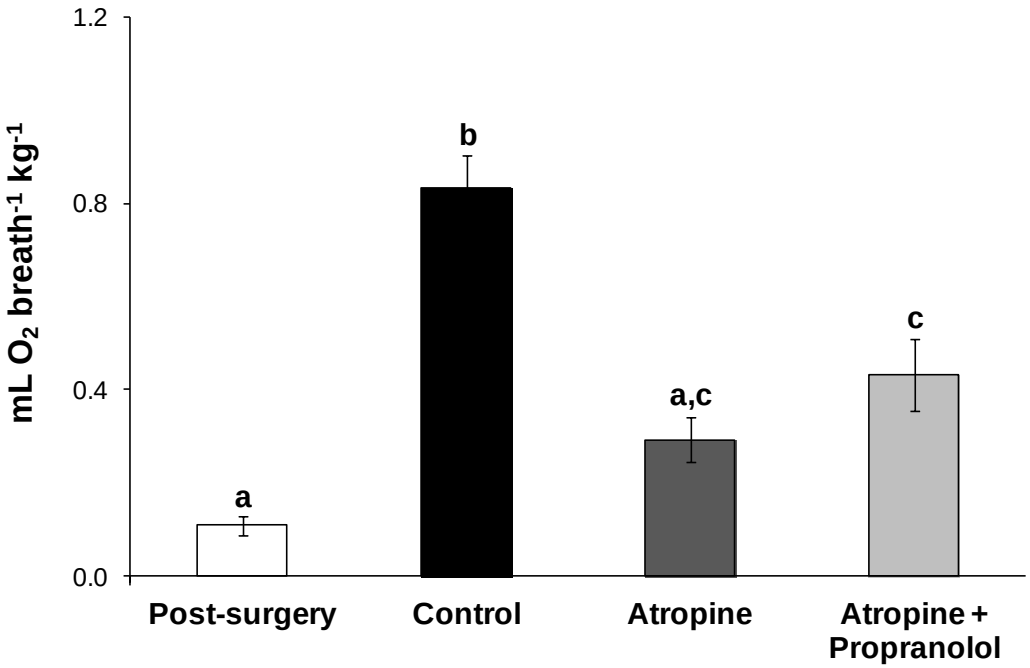

fig. S3. The effect of autonomic modulation of HRV on mean oxygen uptake per breath in *L. paradoxa* at at 25ºC. Mean, mass specific oxygen uptake (per breath) after instrumentation (Post-surgery, n = 8); following 24h of recovery (Control, n = 8); after cholinergic receptor blockade (Atropine, n = 6); total blockade (β-adrenergic and cholinergic receptors) (Atropine + Propranolol, n = 6). The control group shows a clearly elevated oxygen uptake at each breath compared with disturbed (post-surgery) and blocked animals. Data are plotted as means ± S.E.M. Different letters denote significant differences (ANOVA Tukey post-hoc, $P < 0.05$).

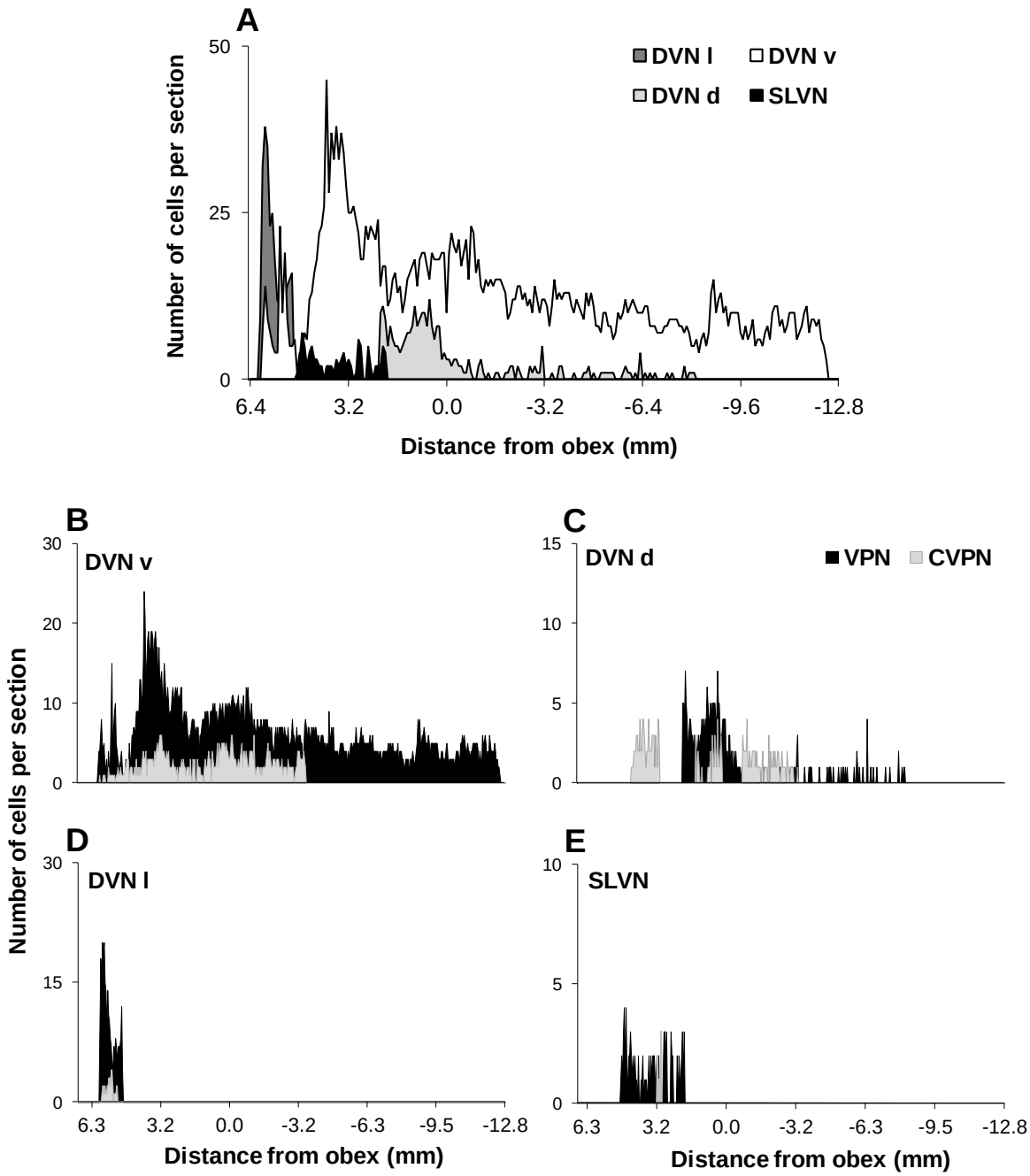

fig. S4. The rostrocaudal distribution of cell bodies of VPN in the brainstem of *L. paradoxa*. Data were taken from fish with the best fills of either the Fluorogold or the DiI retrograde tracers. Neuron numbers per section were plotted against their rostrocaudal distribution in relation to Obex. (**A**) Numbers of vagal preganglionic motoneurones (VPN) stained with Fluorogold, located in different motor nuclei. DVNv denotes the distribution of the major group of VPN located in the ventral DVN; DVNd denotes a smaller group of cells in the dorsal DVN; DVNl denotes more rostral cell group in the DVN; and SLVN denote the scattered distribution of some VPN located laterally outside of the DVN. (**B**, **C**, **D**, and **E**) Overlapping distribution of vagal preganglionic neurons (VPN) and cardiac vagal preganglionic neurones (CVPN) in the different groups of cells (DVNv; DVNd; DNVl; SLVN) identified in the piramboia brainstem.

table S1. Morphometric variables in the ultrastructure of transverse sections of the cardiac branches of the vagus nerve in *L. paradoxa*. Data expressed as mean ± SEM. MF = myelinated fiber, UMF = unmyelinated fibers. Asterisk indicates differences between right and left branches (unpaired Student's t-test, $P < 0.05$).

Parameters	**Cardiac vagal segment**	
	Right	**Left**
Total fibers density (fibers·mm^{-2})	14.0 ± 0.1	18.0 ± 0.3
UMF density (fibers·mm^{-2})	4.0 ± 0.5	3.8 ± 0.1
MF density (fibers·mm^{-2})	9.8 ± 0.5	14.5 ± 2.2*****
UMF diameter (μm)	4.2 ± 0.4	3.0 ± 0.8
Area occupied by UMF (%)	5.1 ± 0.7	5.8 ± 1.7
MF diameter (μm)	11.8 ± 0.4	10.2 ± 0.6
Area occupied by MF (%)	55.8 ± 1.2	64.4 ± 3.1*****
Myelin thickness (μm)	1.6 ± 0.1	1.7 ± 0.1
MF/UMF ratio	2.7 ± 0.3	3.4 ± 0.7