

## Transient increase of C4 burst rate during hypoxic stimulation in the brainstem-spinal cord preparation

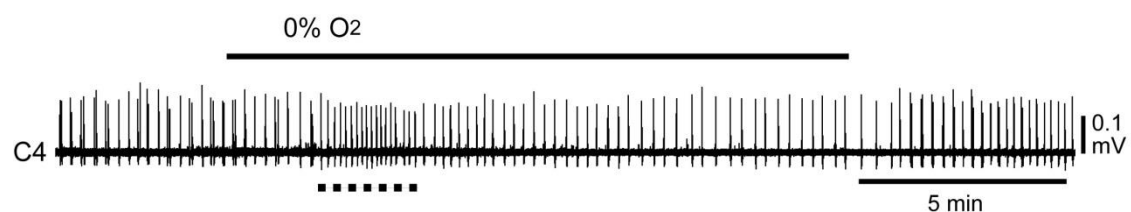
### Methods

Experiments were performed with brainstem-spinal cord preparations from newborn (postnatal day 0-1) Wistar rats. The newborn rats were deeply anesthetized with isoflurane. The brainstem and spinal cord were isolated and superfused at a rate of 3.0 mL/min with the following artificial cerebrospinal fluid (in mM): 124 NaCl, 5.0 KCl, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 2.4 CaCl<sub>2</sub>, 1.3 MgCl<sub>2</sub>, 26 NaHCO<sub>3</sub> and 30 glucose, equilibrated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>, pH 7.4, at 25-26°C. In most experiments, the preparations were cut transversely at a level just rostral to the anterior inferior cerebellar artery. For hypoxic stimulation (15 min), the superfusate was exchanged to 0% O<sub>2</sub> from 95% O<sub>2</sub> at 5% CO<sub>2</sub>.

The initial data analyses were performed using the LabChart 7 Pro software program (ADInstruments, Castle Hill, Australia). The burst rate was calculated from 10 consecutive respiratory cycles. Data are presented as the mean  $\pm$  SD. The significance of values was analyzed by a one-way ANOVA, followed by a Tukey-Kramer multiple comparisons test at a confidence level of  $P < 0.05$  using the GraphPad InStat software program (GraphPad Software Inc., La Jolla, CA, USA).

### Results

We examined C4 burst rate change in response to hypoxic stimulation in the brainstem-spinal cord preparation (n=7). The C4 burst rate was increased at 2-3 min after the start of hypoxic stimulation followed by a decrease:  $3.7 \pm 1.1$ /min in control,  $8.4 \pm 1.3$ /min at 2-3 min after hypoxic stimulation ( $P < 0.001$ , compared to control), and  $4.2 \pm 0.96$ /min after 15 min of the hypoxia. A typical example is shown in Suppl. Fig 1.



Suppl. Figure 1. Transient increase of C4 burst rate during hypoxic stimulation. C4 burst rate was increased at 2-3 min (dotted line) after the start of hypoxic stimulation (0% O<sub>2</sub>) followed by a decrease.