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Short communication

Intermittent but not sustained moderate hypoxia elicits long-term facilitation of hypoglossal motor output

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ABSTRACT

Phrenic long-term facilitation (pLTF) is a form of serotonin-dependent respiratory motor plasticity induced by moderate acute intermittent hypoxia (AIH), but not by moderate acute sustained hypoxia (ASH) of similar cumulative duration. Thus, moderate AIH-induced pLTF is sensitive to the pattern of hypoxia. On the other hand, pLTF induced by severe AIH protocols is neither pattern sensitive nor serotonin dependent (it converts to an adenosine-dependent mechanism). Although moderate AIH also induces hypoglossal LTF (hLTF), no data are available concerning its sensitivity/insensitivity to the pattern of hypoxia. Since hLTF following moderate hypoxia is serotonin-dependent, we hypothesized that hLTF is pattern-sensitive, similar to serotonin-dependent pLTF. Integrated hypoglossal nerve activity was recorded in urethane-anesthetized, vagotomized, paralyzed, and ventilated rats exposed to isocapnic AIH (3, 5 min episodes of 11% O_2) or ASH (a single 25 min episode of 11% O_2). Similar to previous studies of pLTF, hypoglossal motor output was elevated for more than 1 h following AIH (50 ± 20%, $p < 0.01$), but not ASH (–6 ± 9%, $p > 0.05$). Frequency LTF was not observed following either hypoxic exposure. Thus, in agreement with our hypothesis, hypoglossal LTF following moderate AIH is pattern-sensitive, similar to phrenic LTF.

1. Introduction

Patterned stimulation is often more effective than continuous stimulation at inducing plasticity in the central nervous system. For example, stimulus presentations spaced over time are more effective at inducing long term memory formation versus an equal duration continuous presentation (Ebbinghaus, 1913; Beck et al., 2000; Sutton et al., 2002; Cepeda et al., 2006). This phenomenon, known as the "spacing effect," is expressed widely throughout the animal kingdom, and has been extensively studied in several animal models and humans because of its potential utility in education (Kerfoot, 2010), psychology (Goverover et al., 2009b), advertising (Janiszewski et al., 2003; Appleton-Knapp et al., 2005) and physical rehabilitation (Goverover et al., 2009a). Consistent with the spacing effect in learning and memory, patterned stimulation (intermittent vs. continuous) is also more effective at eliciting synaptic plasticity in the nervous system. For example, intermediate-term memory formation and synaptic facilitation in *Aplysia* (Mauelshagen et al., 1998; Sutton et al., 2002), and hippocampal long-term potentiation in rodents (Kauer, 1999; Nguyen et al., 2000; Scharf et al., 2002) exhibit pattern sensitivity, with intermittent more effective than continuous stimulus presentations. Despite its importance to neuroplasticity, mechanisms giving rise to pattern sensitivity are not well known.

Some forms of hypoxia-induced respiratory motor plasticity exhibit clear pattern sensitivity (Baker and Mitchell, 2000; Wilkerson et al., 2008). For example, serotonin-dependent phrenic long-term facilitation (pLTF) can be elicited by moderate acute intermittent (AIH), but not moderate acute sustained hypoxia (ASH) of similar cumulative duration (Mitchell et al., 2001; Baker and Mitchell, 2000). Both severe intermittent and sustained hypoxia also elicit pLTF, although this form of pLTF is mediated by a distinct, adenosine-dependent mechanism (Nichols et al., 2012; Devinney et al., 2016). While episodic spinal serotonin receptor activation is required to elicit phrenic motor facilitation in rats, a single, larger serotonin injection fails to elicit the response (MacFarlane and Mitchell, 2009). Further, whereas intermittent but not sustained serotonin receptor activation elicits phrenic long-term facilitation in neonatal brainstem-spinal cord preparations, similar pattern sensitivity is not observed in inspiratory intercostal activity (Lovett-Barr et al., 2006). Thus, all forms of respiratory motor plasticity do not exhibit similar pattern sensitivity to the inducing stimulus.

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Another form of hypoxia-induced respiratory motor plasticity is hypoglossal long-term facilitation (hLTF; Bach and Mitchell, 1996). Similar to pLTF, hLTF is expressed as a progressive increase in hypoglossal motor output induced by moderate AIH (Bach and Mitchell, 1996; Baker-Herman and Strey, 2011). Hypoglossal LTF might stabilize the upper airways, maintaining upper airway patency during sleep (Fuller, 2005; Mahamed and Mitchell, 2007; Baker-Herman and Strey, 2011). Thus, greater understanding of distinctions between the mechanisms giving rise to pLTF versus hLTF could lead to new therapeutic approaches that minimize certain forms of obstructive sleep apnea (Baker-Herman and Strey, 2011). However, little is known concerning similarities and differences in the mechanisms of pLTF and hLTF, and there are no reports concerning pattern-sensitivity of hLTF with moderate hypoxia. Here, we demonstrate that hLTF exhibits pattern sensitivity, similar to moderate AIH-induced pLTF.

2. Materials and methods

Experiments were performed on 3–5 month old male Sprague-Dawley rats (colony PO4, Charles River Inc., Wilmington, MA). Rats were individually housed in a controlled environment (12 h light/dark cycle), with food and water ad libitum. The University of Wisconsin, School of Veterinary Medicine Animal Care and Use Committee approved all protocols.

2.1. Surgical preparation and nerve isolation

Rats were initially anesthetized in a closed chamber containing isoflurane followed by isoflurane administration through a nose cone (3.0 – 3.5% in 50% O_2, balance N_2). The trachea was cannulated to enable pump-ventilation (tidal volume, 2–2.5 mL; $FIO_2 = 0.50$; Rodent Respirator model 682, Harvard Apparatus, South Natick, MA). A bilateral vagotomy was performed at the mid-cervical level to prevent entrainment of respiratory motor output with the ventilator. Catheters were placed into the tail vein for fluid administration (1:11 by volume $NaHCO_3$:lactated Ringer's; 2.5 mL/hr) and the femoral artery for blood pressure measurement and to draw blood samples for blood gas analysis. Body temperature was maintained at 37.5 ± 1.0 °C using a rectal probe and custom-designed heated table. The left hypoglossal nerve was isolated using a dorsal approach, cut distally, desheathed and placed on a bipolar silver electrode. Rats were slowly converted to urethane anesthesia (1.6 g/kg, i.v.) and then paralyzed with pancuronium bromide to prevent spontaneous breathing movements (2.5 mg/kg, i.v., supplemented as necessary). End-tidal CO_2 was measured throughout the experiment using a flow-through capnograph (Capnogard, Model 1265, Novametrix; Wallingford, CT) with sufficient response time to measure expiratory gases in rats.

2.2. Experimental protocols

The CO_2 apneic threshold was determined by decreasing CO_2 levels and/or increasing the ventilator rate until nerve activity ceased. Inspired CO_2 levels were then increased; the end-tidal CO_2 at which phrenic activity resumed was taken as the recruitment threshold (Mahamed and Mitchell, 2007). End-tidal CO_2 levels were then set 1–2 mm Hg above the recruitment threshold. A stable hypoglossal neurogram was established and an initial blood sample was taken to establish baseline PaO_2, $PaCO_2$, pH, and base excess values (0.3 mL in 0.5 mL heparinized glass syringe; ABL-500, Radiometer, Copenhagen, Denmark; unused blood was returned to the animal). Rats were given 3, 5 min episodes of hypoxia (i.e., AIH; $FIO_2 = 0.11 \pm 0.1$, $PaO_2 = 39 \pm 1$ mm Hg), separated by 5 min of baseline conditions ($FIO_2 = 0.5$, $PaO_2 > 250$ mm Hg), or a single, cumulative 25 min hypoxic episode (i.e., ASH; $FIO_2 = 0.11 \pm 0.1$, $PaO_2 = 38 \pm 1$ mm Hg). Hypoglossal activity was monitored 60 min post-hypoxia to determine LTF magnitude. Arterial blood samples were drawn and analyzed during the final minute of the first hypoxic episode, and 15, 30 and 60 min after the final hypoxic episode. Additional rats that did not receive hypoxia (time controls) were used to verify the stability of nerve output over a similar time period in this preparation. Throughout the protocol, isocapnic conditions (± 1 mm Hg from baseline $PaCO_2$) were maintained by adjusting ventilator frequency and/or inspired CO_2.

2.3. Electrophysiological methods

Hypoglossal nerve activity was amplified (x 10,000), band pass filtered (100 Hz to 10 kHz; Model 1700, A-M Systems, Inc., Carlsborg, WA), and integrated (time constant = 50 ms, Model MA-821RSP, CWE Inc., Ardmore, PA). Integrated signals were digitized and processed with commercially available software (WINDAQ software, DATAQ Instruments, Akron, OH). Peak integrated hypoglossal burst amplitude, burst frequency, and mean arterial blood pressure were calculated over a 60 s period just prior to the first hypoxic episode (baseline), at the end of the first hypoxic episode or the equivalent time point during sustained hypoxia (short-term hypoxic response), and 30 and 60 min post-hypoxia. Data were included in the analysis only if isocapnic conditions were successfully maintained. Amplitude data are expressed as the change in hypoglossal burst amplitude, expressed as a percent change from baseline values. Frequency data are reported as a change from baseline in bursts per minute (delta burst frequency). Data were compared using a one-way ANOVA or two-way ANOVA with repeated measures design as applicable (Fisher LSD post-hoc test; SigmaStat 2.03, SPSS Inc., Chicago, IL).

3. Results

3.1. Physiological variables

No significant differences were observed in the CO_2 recruitment threshold for rats treated with (IH: 41 ± 1 mmHg, n = 9; SH: 40 ± 1 mmHg, n = 14) or without hypoxia (42 ± 1 mmHg, n = 14; $p > 0.05$). Under baseline conditions, mean arterial blood pressure was not different between treatment groups (IH: 112 ± 5 mmHg, SH: 110 ± 4 mmHg; Table 1; $p > 0.05$), or time controls that did not receive hypoxia: (112 ± 5 mmHg; Table 1, $p > 0.05$). Similar to other studies from our laboratory, mean arterial blood pressure significantly decreased during the hypoxic stimulus versus baseline (IH: 62 ± 4 mmHg, SH: 65 ± 7 mmHg; $p < 0.05$), but not in time controls (112 ± 5 mmHg; Table 1, $p > 0.05$). We did not observe

Table 1
Temporal changes in mean arterial blood pressure (MABP), $PaCO_2$, and PaO_2 in rats.

Treatment	N	Baseline	1st HX	60 min post-HX
No hypoxia	14	112 ± 5 (MABP) 47.7 ± 1.2 ($PaCO_2$) 267.2 ± 4.0 (PaO_2)	112 ± 5 47.7 ± 1.2 265.9 ± 4.7	105 ± 5 47.6 ± 1.2 252.2 ± 8.7
Intermittent hypoxia	9	112 ± 5 45.2 ± 1.2 275.7 ± 6.5	68 ± 4* 44.0 ± 1.7 38.9 ± 0.8*	105 ± 4* m45.9 ± 1.1 262.0 ± 8.1
Sustained hypoxia	14	110 ± 4 44.4 ± 1.8 257.0 ± 4.9	65 ± 7* 43.9 ± 0.8 37.9 ± 1.2*	100 ± 3*44.0 ± 0.9 245.4 ± 8.0

Relative to baseline, mean arterial blood pressure (MABP), arterial partial pressure of CO_2 ($PaCO_2$), and arterial partial pressure of O_2 (PaO_2) were not significantly different over time in rats that did not receive hypoxia ($p > 0.05$). As expected, PaO_2 significantly decreased during hypoxia exposure ($P < 0.05$), but returned to baseline values following exposure. Rats treated with intermittent or sustained hypoxia showed similar, significant decreases in MABP during hypoxia ($p < 0.05$) and small but significant decreases in MABP 60 min post-hypoxia compared to baseline ($p < 0.05$). Overall, there was not a significant treatment effect on MABP ($p > 0.05$). *Significantly different from baseline within treatment group.