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Combined hypoxia and hypercapnia, but not hypoxia alone, suppresses neurotransmission from orexin to hypothalamic paraventricular spinally-projecting neurons in weanling rats

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Abstract

Both orexin neurons in the lateral hypothalamus and spinally-projecting pre-sympathetic neurons (PSNs) in the paraventricular nucleus of the hypothalamus (PVN) play an important role in the regulation of cardiovascular function under normal conditions and during cardiovascular challenges such as hypoxia and/or hypercapnia. We have previously established, using selective optogenetic excitation of orexin neurons and pathways, there is a heterogeneous neurotransmission from orexin neurons to PSNs in the PVN. This study was undertaken to test whether this pathway is altered by acute exposure to hypoxia alone and/or combined hypoxia and hypercapnia (H/H). To test this hypothesis, we selectively expressed channelrhodopsin-2 (ChR2) in orexin neurons in the lateral hypothalamus and photoactivated ChR2-expressing fibers to evoke postsynaptic currents in spinally-projecting PSNs in an in-vitro slice preparation in rats. In accordance with previously published data, two subpopulations of spinally-projecting PSNs were established, including those with glutamatergic or GABAergic inputs from orexin neurons. Hypoxia alone did not alter the peak amplitude of either glutamatergic or GABAergic neurotransmission, however, H/H significantly inhibited both glutamatergic and GABAergic neurotransmission from orexin neurons to SPNs. In conclusion, H/H may modulate cardiovascular function by affecting heterogeneous pathways from orexin neurons to spinally-projecting PSNs in the PVN.

Keywords

orexin; sympathetic; neurons; optogenetic; hypoxia; hypercapnia

1. INTRODUCTION

We have previously established, using selective optogenetic excitation of orexin neurons and pathways, there is a heterogeneous neurotransmission from orexin neurons to pre-sympathetic bulbospinal neurons (PSNs) in the paraventricular nucleus of the hypothalamus

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(PVN) (Dergacheva et al., 2017). PSNs in the PVN project directly to the intermediolateral cell column of the thoracic spinal cord with collaterals to the rostral ventrolateral medulla and play an important role in the cardiovascular sympathoactivation (Hosoya et al., 1991; Kc and Dick, 2010; Ranson et al., 1998; Sawchenko and Swanson, 1982). Orexin neurons in the lateral hypothalamus are involved in regulation of many physiological functions including autonomic function, arousal, sleep, feeding, ventilatory chemosensitivity and breathing (Branch et al., 2016; Burdakov et al., 2003; Deng et al., 2007; Dergacheva et al., 2013; Dergacheva, 2015; Nattie and Li, 2012; van den Pol et al., 1998; Wu et al., 2002; Yamanaka, 2015; Young et al., 2005). The results from our previous study suggest that orexin neurons may exert sympathoexcitatory control of cardiovascular function upon activation of the direct projection from orexin neurons to PSNs in the PVN (Dergacheva et al., 2017).

It has been recently shown that orexin neurons are sensitive to hypoxia alone and a combination of hypoxia and hypercapnia (H/H) (Dergacheva et al., 2016). In addition, the neurotransmission from orexin neurons to parasympathetic cardiac vagal neurons is compromised with chronic exposure to H/H (Dergacheva, 2015). We propose that hypoxia and/or H/H may also affect the synaptic pathway from orexin neurons to PSNs in the PVN and these neurophysiological mechanisms may contribute to sympathetic and cardiovascular dysfunction associated with diseases characterized by repetitive breathing cessations resulting in H/H such as obstructive sleep apnea (OSA) (Bradley et al., 1986; Guillemineault et al., 1976).

Thus, this study was undertaken to test the hypothesis that acute hypoxia and/or H/H alters the synaptic neurotransmission from orexin neurons to PSNs in the PVN. To accomplish this goal, we used transgenic orexin Cre-rats to express channelrhodopsin-2 (ChR2) in orexin neurons and fibers. Postsynaptic currents in fluorescently identified PSNs were recorded upon photoactivation of ChR2 expressing orexin fibers that surrounded these neurons in an in-vitro slice preparation before, during and post exposure to hypoxia alone and, in another set of experiments, to combined H/H.

2. RESULTS

2.1. Monosynaptic projections from orexin-containing neurons to PSNs in the PVN

Photostimulation of ChR2-expressing fibers surrounding PSNs in the PVN generated fast postsynaptic responses in PSNs. The average latency of the responses were 4.1 ± 0.3 ms (ranging from 2.3 to 6.9 ms, $n = 24$ neurons) indicating a monosynaptic connection (Petreanu et al., 2009; Pinol et al., 2012). In agreement with our previous data (Dergacheva et al., 2017), these light-triggered responses included glutamatergic excitatory postsynaptic currents (EPSCs) which were blocked with CNQX (50 μ M) and GABAergic inhibitory postsynaptic currents (IPSCs) which were abolished by application of gabazine (25 μ M).

2.2. Acute hypoxia did not change synaptic neurotransmission from orexin neurons to PSNs

First we investigated the effects of acute hypoxia on the light-triggered postsynaptic neurotransmission to PSNs. Slices were exposed to hypoxia by changing control aCSF

equilibrated with 95% O₂–5% CO₂, pH 7.4 to an identical solution equilibrated with 90% N₂–5% O₂–5% CO₂, pH 7.4. During 10-min exposure to acute hypoxia no significant alterations occurred in either peak amplitude of glutamatergic EPSCs ($P>0.05$, 1-way repeated-measures ANOVA and Dunnett's posttest, $n = 6$, Fig. 1, top) or peak amplitude of GABAergic IPSCs ($P>0.05$, 1-way repeated-measures ANOVA and Dunnett's posttest, $n = 6$, Fig. 1, bottom).

2.3. H/H inhibited synaptic neurotransmission from orexin neurons to PSNs

We next investigated whether H/H, elicited by equilibrating aCSF with 85% N₂, 6% O₂, and 9% CO₂, pH 7.1, affected the amplitude of light-triggered postsynaptic currents in PSNs. H/H produced a significant depression of the peak amplitude of glutamatergic EPSCs (from 56.2 ± 11 pA to 27.9 ± 4.7 pA, H/H at 9–10 min, $n=6$, $P<0.05$, 1-way repeated-measures ANOVA and Dunnett's posttest, $n = 6$, Fig. 1, top). Similarly, the peak amplitude of GABAergic IPSCs was significantly diminished by H/H (from 83.8 ± 14 pA to 52.5 ± 15 pA, H/H at 9–10 min; $P<0.05$, 1-way repeated-measures ANOVA and Dunnett's posttest, $n = 6$, Fig. 2, bottom). The amplitude of both glutamatergic and GABAergic postsynaptic currents recovered upon 9–10 min of reoxygenation (56.2 ± 11 pA vs. 51.2 ± 14 pA, $P>0.05$ $n = 6$ and 83.8 ± 14 pA vs. 81.8 ± 22 pA, $P>0.05$, $n = 6$, respectively, see Fig. 2, top and bottom, respectively).

3. DISCUSSION

There are two major findings in this study: 1) Photoactivation of ChR2-expressing orexinergic fibers in the PVN yields short-latency heterogeneous responses in PSNs including glutamatergic EPSCs and GABAergic IPSCs, which are not changed by hypoxia. 2) However, combined H/H significantly diminishes both glutamatergic and GABAergic postsynaptic neurotransmission from orexin neurons to PSNs in the PVN.

Systemic hypoxia (10% O₂) does not change activity of putative PSNs in the PVN that project to the rostral ventrolateral medulla (RVLM) (Hirooka et al., 1997). Similar results were obtained by other authors, which have shown that acute hypoxia (10%) activates neuroendocrine, but not spinally-projecting PSNs in the PVN (Coldren et al., 2017). These previous results are in consensus with the current findings demonstrating that hypoxia alone does not affect synaptic pathway from orexin to PSNs. In contrast, combined H/H inhibits neurotransmission from orexin neurons to PSNs. We have previously shown that H/H has more profound depressor effects on the firing activity of orexin neurons than hypoxia alone (Dergacheva et al., 2016). We propose that profound depression of firing activity of orexin neurons with H/H may contribute to the inhibition of neurotransmission from orexin neurons to PSNs. Consistent with the results from this study, both orexin receptor expressions (orexin receptors 1 and 2) are increased in the PVN after intermittent exposure to H/H (Hunt et al., 2013). The authors speculated that upregulation of these orexin receptors is the result of decreased level of orexin release and receptor density compensation due to H/H exposures (Du et al., 2016). Similar results have been obtained with acute exposure to hypercapnia alone. Orexin mRNA levels are decreased whereas the mRNA levels of orexin receptor 1 are increased in the PVN after 3 hour exposure to hypercapnia (Wang et al., 2013).

Hypoxia or H/H typically simulate neurons in the brain regions involved in cardiorespiratory regulation (Kramer et al., 1999). For instance, neurons in caudal hypothalamus are activated by hypoxia, hypercapnia, as well as combination of H/H (Dillon and Waldrop, 1992; Dillon and Waldrop, 1993). Similarly, the firing rates of neurons in the periaqueductal gray are increased by hypoxia or hypercapnia (Kramer et al., 1999). Combined H/H elicits a significant increase in the firing discharge of more than 50% neurons in the ventrolateral medulla (Nolan and Waldrop, 1993). Neuronal activation in these brain regions, involved in cardiorespiratory regulation, likely contributes to the generation of cardiorespiratory activation in response to hypoxia or hypercapnia. In contrast, orexin neurons in the lateral hypothalamus are depressed by acute H/H (Dergacheva et al., 2016). Moreover, the results from this study indicate that the pathway from orexin neurons to PSNs in the PVN is inhibited by H/H. Interestingly, another important pathway from hypoxia-sensitive neurons in the caudal ventrolateral medulla to PVN neurons is likely activated by hypoxia (King et al., 2013). Taking the results from previous studies and data from this work together, we speculate that H/H-inhibited neurotransmission from orexin to PSNs in the PVN could be a path-specific alteration.

The results from this study indicate that both light-triggered glutamatergic EPSCs and GABAergic IPSCs in PSNs are inhibited by H/H exposure. Glutamatergic neurotransmission tends to decrease as early as at 2 minutes of H/H exposure, however a significant decrease only occurs at 10 min of the exposure. In contrast, very small alteration in GABAergic IPSCs occurs at 2 min of H/H. Orexinergic signaling has been shown to facilitate glutamatergic, but not GABAergic, neurotransmission from orexin neurons to PSNs (Dergacheva et al., 2017). Inhibition of orexinergic transmission with H/H may contribute to the effects of H/H on glutamatergic EPSCs in PSNs. It is worth mentioning that these in-vitro results were obtained at room temperature, which may increase the delay of the responses. It is possible that in a whole-animal model other mechanisms (including peripheral chemoreceptors) contribute to the effects of H/H that build more rapidly to evoke responses. It is likely that different subpopulations of PSNs, including those with excitatory and inhibitory inputs from orexin-containing neurons, may play differential roles in sympathetic cardiovascular responses to H/H. Subpopulation of PSNs with GABAergic projections from orexin neurons may contribute to the increased sympathetic nerve discharge in response to acute hypoxia or hypercapnia (Fukuda et al., 1989). Subpopulation of PSNs that receive excitatory glutamatergic projection from orexin neurons may play a role in the arousal deficits observed in OSA patients (Harrington et al., 2002) and animals exposed to H/H (Waters and Tinworth, 2005). A majority of spinally-projecting PSNs in the PVN input into neurons in the rostral ventrolateral medulla (Hosoya et al., 1991; Kc and Dick, 2010; Ranson et al., 1998; Sawchenko and Swanson, 1982) that constitute pre-autonomic sympathetic network involved in the control of cardiovascular function and arousal (Burke et al., 2014; Chen et al., 2013). H/H-elicited inhibition of excitatory glutamatergic neurotransmission from orexin neurons to a subpopulation of PSNs may diminish output sympathetic activity involved in arousal.

In conclusion, H/H diminishes both excitatory and inhibitory synaptic neurotransmission from orexin neurons to PSNs in the PVN. This hypothalamic mechanism may play a role in

cardiovascular responses to H/H as well as to cardiovascular abnormalities associated with OSA.

4. EXPERIMENTAL PROCEDURES (Branch et al., 2016)

4.1. Animals and ethical approval

Both sexes of transgenic orexin-EGFP-2A-Cre rats were used in this study. The generation of these animals has been described previously (Dergacheva et al., 2016b, 2017). All animal procedures were performed in compliance with the institutional guidelines at George Washington University and are in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to reduce animal discomfort and minimize number of rats used in this study.

4.2. PSNs labeling

Labeling PSNs in the PVN, performed in this study, has been described previously (Hosoya et al., 1991, Womack et al., 2006, Dergacheva et al., 2013, Dergacheva et al., 2017). Orexin-Cre rats (postnatal day 3) were anesthetized with hypothermia. The upper thoracic spinal cord was exposed, and the retrograde tracer cholera toxin subunit B conjugated with Alexa Fluor 555 (1%, Invitrogen, Carlsbad, CA) was injected bilaterally into T2-T4 spinal segments (total volume, 50 nl).

4.3. Viral injections into the lateral hypothalamus

Adeno-associated viral vector with “FLEX-switch” ChR2 construct [AAV1.EF1a.DIO.hChR2(H134R)-EYFP.WPRE.hGH, Catalog No. AV-1–20298P Penn Vector Core, Philadelphia, PA] was injected into the lateral hypothalamus of orexin-Cre rats as previously described (Dergacheva et al., 2016a, 2017). Injections were made while the animals were anesthetized with hypothermia and mounted in a stereotactic apparatus with a neonatal adapter (Stoelting, Wood Dale, IL). The skull was exposed and a small burr hole was made to position a pulled calibrated pipette (VWR, Radnor, PA) containing viral vector at the following coordinates: 1.7–1.9 mm posterior and 0.4 mm lateral relative to bregma. The pipette tip was lowered 5.2 mm from the dorsal surface of the brain and 50 nl of viral vector was slowly injected. The pipette was left in place for 5 minutes, before retraction.

4.4. Slice preparation and electrophysiology

On the day of experiment, animals (21–24 days old) were anesthetized with isoflurane, and glycerol-based artificial cerebrospinal fluid (aCSF) was perfused transcardially. Glycerol-based aCSF (4°C) contained (in mM): 252 glycerol, 1.6 KCl, 1.2 NaH₂PO₄, 1.2 MgCl₂, 2.4 CaCl₂, 26 NaHCO₃, and 11 glucose. The brain was removed, and 300-µm-thick coronal slices of the hypothalamus were obtained with a vibratome. The slices were transferred to a solution of the following composition (in mM) 110 N-methyl-D-glucamine, 2.5 KCl, 1.2 NaH₂PO₄, 25 NaHCO₃, 25 glucose, 0.5 CaCl₂, and 10 mM MgSO₄ equilibrated with 95% O₂-5% CO₂ (pH 7.4) at 34°C for 15 min. The slices were then transferred to a recording chamber, which allowed perfusion (5–10 ml/min) of aCSF at room temperature (25°C) containing (in mM) 125 NaCl, 3 KCl, 2 CaCl₂, 26 NaHCO₃, 5 glucose, and 5 HEPES

equilibrated with 95% O₂-5% CO₂ (pH 7.4) for at least 30 min before experiments were conducted.

Individual PSNs were identified in the PVN by the presence of the fluorescent tracer. These identified cells were then imaged with differential interference contrast optics, infrared illumination, and infrared-sensitive video detection cameras to gain better spatial resolution. Postsynaptic neurotransmission to PSNs were studied in a voltage-clamp configuration at a holding potential of -80 mV with an Axopatch 200 B and pClamp 8 software (Axon Instruments, Union City, CA). Patch pipettes (2.5–3.5 MΩ) were filled with a solution consisting of (in mM) 150 KCl, 2 MgCl₂, 2 EGTA, 10 HEPES, and 2 Mg-ATP, pH 7.3. All drugs used in this electrophysiological study were purchased from Sigma-Aldrich Chemical (St. Louis, MO).

Selective photostimulation of ChR2-expressing fibers surrounding PSNs was performed by using a 473-nm blue laser light (CrystaLaser, Reno, NV). The laser was attached to the microscope with a dual housing adapter (Nikon). A series of 60 consecutive single stimulations (3-ms duration, at 1 Hz) were applied to each neuron studied. Laser light intensity was kept at an output of 10 mW.

4.5. Hypoxia and H/H exposures

Slices were exposed to acute hypoxia by changing control aCSF equilibrated with 95% O₂-5% CO₂, pH 7.4 to an identical solution equilibrated with 90% N₂-5% O₂-5% CO₂, pH 7.4. Slices were exposed to acute H/H by changing control aCSF to an identical solution, equilibrated with 85% N₂-6% O₂-9% CO₂, pH 7.1. Slices were exposed to acute hypoxia or H/H for 10 min, and then slices were re-oxygenated over 10 min by returning the perfusate to the control aCSF.

4.6. Data Analysis and Statistics

Postsynaptic currents were measured using the pClamp 8 software (Molecular Devices, Sunnyvale, CA). A series of 60 consecutive single stimulations were applied 2 minutes before hypoxia or H/H exposure, at 1, 4, and 9 minutes of the exposure and at 9 minute post exposure. The responses to a series of 60 consecutive photostimulations in each neuron were averaged in all series of experiments. The mean value from each neuron in the population was averaged for the population of neurons to create a summary of results for each condition. Results are presented as mean ± SE and statistically compared using GraphPad Prism 5 software and ANOVA with repeated measures and Dunnett's posttest. Significant difference was set at $p < 0.05$. Only one experiment was performed from each animal. In total, 24 neurons from 24 rats were studied.

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ABBREVIATIONS

aCSF	Artificial cerebrospinal fluid
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ChR2	channelrhodopsin-2
H/H	hypoxia and hypercapnia
EPSCs	excitatory postsynaptic currents
IPSCs	inhibitory postsynaptic currents
PSNs	pre-sympathetic neurons

References

- Bradley TD, Rutherford R, Lue F, Moldofsky H, Grossman RF, Zamel N, Phillipson EA. Role of diffuse airway obstruction in the hypercapnia of obstructive sleep apnea. *Am Rev Respir Dis.* 1986; 134:920–4. [PubMed: 3777688]
- Branch AF, Navidi W, Tabuchi S, Terao A, Yamanaka A, Scammell TE, Diniz Behn C. Progressive Loss of the Orexin Neurons Reveals Dual Effects on Wakefulness. *Sleep.* 2016; 39:369–77. [PubMed: 26446125]
- Burdakov D, Liss B, Ashcroft FM. Orexin excites GABAergic neurons of the arcuate nucleus by activating the sodium–calcium exchanger. *J Neurosci.* 2003; 23:4951–7. [PubMed: 12832517]
- Burke PG, Abbott SB, Coates MB, Viar KE, Stornetta RL, Guyenet PG. Optogenetic stimulation of adrenergic C1 neurons causes sleep state-dependent cardiorespiratory stimulation and arousal with sighs in rats. *Am J Respir Crit Care Med.* 2014; 190:1301–10. [PubMed: 25325789]
- Chen CY, Kuo TB, Hsieh IT, Yang CC. Electrical stimulation of the rostral ventrolateral medulla promotes wakefulness in rats. *Sleep Med.* 2013; 14:1076–84. [PubMed: 24047536]
- Coldren KM, Li DP, Kline DD, Hasser EM, Heesch CM. Acute hypoxia activates neuroendocrine, but not presympathetic, neurons in the paraventricular nucleus of the hypothalamus: differential role of nitric oxide. *Am J Physiol Regul Integr Comp Physiol.* 2017; 312:R982–R995. [PubMed: 28404583]
- Deng BS, Nakamura A, Zhang W, Yanagisawa M, Fukuda Y, Kuwaki T. Contribution of orexin in hypercapnic chemoreflex: evidence from genetic and pharmacological disruption and supplementation studies in mice. *J Appl Physiol* (1985). 2007; 103:1772–9. [PubMed: 17717124]
- Dergacheva O, Boychuk CR, Mendelowitz D. Developmental changes in GABAergic neurotransmission to presympathetic and cardiac parasympathetic neurons in the brainstem. *J Neurophysiol.* 2013; 110:672–9. [PubMed: 23657280]
- Dergacheva O. Chronic intermittent hypoxia alters neurotransmission from lateral paragigantocellular nucleus to parasympathetic cardiac neurons in the brain stem. *J Neurophysiol.* 2015; 113:380–9. [PubMed: 25318765]
- Dergacheva O, Yamanaka A, Schwartz AR, Polotsky VY, Mendelowitz D. Hypoxia and hypercapnia inhibit hypothalamic orexin neurons in rats. *J Neurophysiol.* 2016; 116:2250–2259. [PubMed: 27559138]
- Dergacheva O, Yamanaka A, Schwartz AR, Polotsky VY, Mendelowitz D. Optogenetic identification of hypothalamic orexin neuron projections to paraventricular spinally projecting neurons. *Am J Physiol Heart Circ Physiol.* 2017; 312:H808–H817. [PubMed: 28159808]
- Dillon GH, Waldrop TG. In vitro responses of caudal hypothalamic neurons to hypoxia and hypercapnia. *Neuroscience.* 1992; 51:941–50. [PubMed: 1336828]
- Dillon GH, Waldrop TG. Responses of feline caudal hypothalamic cardiorespiratory neurons to hypoxia and hypercapnia. *Exp Brain Res.* 1993; 96:260–72. [PubMed: 8270021]
- Du MK, Hunt NJ, Waters KA, Machaalani R. Cumulative effects of repetitive intermittent hypercapnic hypoxia on orexin in the developing piglet hypothalamus. *Int J Dev Neurosci.* 2016; 48:1–8. [PubMed: 26548856]
- Fukuda Y, Sato A, Suzuki A, Trzebski A. Autonomic nerve and cardiovascular responses to changing blood oxygen and carbon dioxide levels in the rat. *J Auton Nerv Syst.* 1989; 28:61–74. [PubMed: 2511237]

- Guilleminault C, Tilkian A, Dement WC. The sleep apnea syndromes. *Annu Rev Med.* 1976; 27:465–84. [PubMed: 180875]
- Harrington C, Kirjavainen T, Teng A, Sullivan CE. Altered autonomic function and reduced arousability in apparent life-threatening event infants with obstructive sleep apnea. *Am J Respir Crit Care Med.* 2002; 165:1048–54. [PubMed: 11956043]
- Hirooka Y, Polson JW, Potts PD, Dampney RA. Hypoxia-induced Fos expression in neurons projecting to the pressor region in the rostral ventrolateral medulla. *Neuroscience.* 1997; 80:1209–24. [PubMed: 9284071]
- Hosoya Y, Sugiura Y, Okado N, Loewy AD, Kohno K. Descending input from the hypothalamic paraventricular nucleus to sympathetic preganglionic neurons in the rat. *Exp Brain Res.* 1991; 85:10–20. [PubMed: 1884750]
- Hunt NJ, Waters KA, Machaalani R. Orexin receptors in the developing piglet hypothalamus, and effects of nicotine and intermittent hypercapnic hypoxia exposures. *Brain Res.* 2013; 1508:73–82. [PubMed: 23500635]
- Kc P, Dick TE. Modulation of cardiorespiratory function mediated by the paraventricular nucleus. *Respir Physiol Neurobiol.* 2010; 174:55–64. [PubMed: 20708107]
- King TL, Kline DD, Ruyle BC, Heesch CM, Hasser EM. Acute systemic hypoxia activates hypothalamic paraventricular nucleus-projecting catecholaminergic neurons in the caudal ventrolateral medulla. *Am J Physiol Regul Integr Comp Physiol.* 2013; 305:R1112–23. [PubMed: 24049118]
- Kramer JM, Nolan PC, Waldrop TG. In vitro responses of neurons in the periaqueductal gray to hypoxia and hypercapnia. *Brain Res.* 1999; 835:197–203. [PubMed: 10415374]
- Nattie E, Li A. Respiration and autonomic regulation and orexin. *Prog Brain Res.* 2012; 198:25–46. [PubMed: 22813968]
- Nolan PC, Waldrop TG. In vivo and in vitro responses of neurons in the ventrolateral medulla to hypoxia. *Brain Res.* 1993; 630:101–14. [PubMed: 8118678]
- Petreanu L, Mao T, Sternson SM, Svoboda K. The subcellular organization of neocortical excitatory connections. *Nature.* 2009; 457:1142–5. [PubMed: 19151697]
- Pinol RA, Bateman R, Mendelowitz D. Optogenetic approaches to characterize the long-range synaptic pathways from the hypothalamus to brain stem autonomic nuclei. *J Neurosci Methods.* 2012; 210:238–46. [PubMed: 22890236]
- Ranson RN, Motawei K, Pyner S, Coote JH. The paraventricular nucleus of the hypothalamus sends efferents to the spinal cord of the rat that closely appose sympathetic preganglionic neurones projecting to the stellate ganglion. *Exp Brain Res.* 1998; 120:164–72. [PubMed: 9629958]
- Sawchenko PE, Swanson LW. Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. *J Comp Neurol.* 1982; 205:260–72. [PubMed: 6122696]
- van den Pol AN, Gao XB, Obrietan K, Kilduff TS, Belousov AB. Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. *J Neurosci.* 1998; 18:7962–71. [PubMed: 9742163]
- Wang W, Li Q, Pan Y, Zhu D, Wang L. Influence of hypercapnia on the synthesis of neuropeptides and their receptors in murine brain. *Respirology.* 2013; 18:102–7. [PubMed: 22882587]
- Waters KA, Tinworth KD. Habituation of arousal responses after intermittent hypercapnic hypoxia in piglets. *Am J Respir Crit Care Med.* 2005; 171:1305–11. [PubMed: 15735061]
- Wu M, Zhang Z, Leranath C, Xu C, van den Pol AN, Alreja M. Hypocretin increases impulse flow in the septohippocampal GABAergic pathway: implications for arousal via a mechanism of hippocampal disinhibition. *J Neurosci.* 2002; 22:7754–65. [PubMed: 12196599]
- Yamanaka A. Controlling sleep/wakefulness using optogenetics. *Nihon Shinkei Seishin Yakurigaku Zasshi.* 2015; 35:97–102. [PubMed: 26434098]
- Young JK, Wu M, Manaye KF, Kc P, Allard JS, Mack SO, Haxhiu MA. Orexin stimulates breathing via medullary and spinal pathways. *J Appl Physiol (1985).* 2005; 98:1387–95. [PubMed: 15557013]

Highlights

- Photoactivation of orexin fibers by channelrhodopsin-2 expression
- Hypothalamic pre-sympathetic neurons receive direct projections from orexin neurons
- Hypoxia does not change neurotransmission from orexin to pre-sympathetic neurons
- In contrast, combined hypoxia and hypercapnia inhibits this neurotransmission

Figure 1.

Acute hypoxia did not alter synaptic neurotransmission from orexin neurons to PSNs. As shown in a typical experiment (A) and in the summary data (B), hypoxia did not elicit any significant changes in the peak amplitude of glutamatergic excitatory postsynaptic current (EPSC) evoked by optogenetic stimulation of orexin fibers (n=6 neurons). Similarly, the peak amplitude of GABAergic inhibitory postsynaptic current (IPSC) was unchanged by exposure to acute hypoxia. An experiment from a single neuron that receive GABAergic input is shown in C while the summary data (n=6 neurons) are demonstrated in D. Traces represent average from 60 sweeps. Arrow indicates laser photostimulation in this and next figure.

Figure 2.

Acute hypoxia and hypercapnia (H/H) suppresses synaptic neurotransmission from orexin neurons to PSNs. As demonstrated in a typical experiment (A) and in the summary data (B), 10-min H/H elicited reversible depression of the peak amplitude of glutamatergic excitatory postsynaptic current (EPSC) evoked by optogenetic stimulation of orexin fibers ($n=6$ neurons). Traces in C represent optogenetically-evoked GABAergic inhibitory postsynaptic current (IPSC) recorded from a single PSN. The summary data are shown in D. Similar to EPSCs, 10-min H/H reversibly diminished the peak amplitude GABAergic current in PSNs ($n=6$ neurons). Asterisk indicates statistically significant differences ($*P<0.05$).