Published in final edited form as:

J Chem Neuroanat. 2009 March ; 37(2): 112–117. doi:10.1016/j.jchemneu.2008.12.002.

G Proteins in Rat Prefrontal Cortex (PFC) are Differentially Activated as a Function of Oxygen Status and PFC Region

V.S. Hambrecht¹, P.E. Vlisides¹, B.W. Row², D. Gozal², H.A. Baghdoyan¹, and R. Lydic¹

¹Department of Anesthesiology, University of Michigan, Ann Arbor, MI, USA

²Department of Pediatrics, Comer Children's Hospital, The University of Chicago, Chicago, IL, USA

Abstract

This study tested the hypothesis that activation of guanine nucleotide binding (G) proteins in rat prefrontal cortex (PFC) is altered by hypoxia. G protein activation by the cholinergic agonist carbachol and the opioid agonist DAMGO was quantified using [³⁵S]GTPγS autoradiography. G proteins were expressed as nCi/g tissue in the PFC of 18 rats exposed for 14 consecutive days to sustained hypoxia (10 % O₂), intermittent hypoxia (10 % and 21 % O₂ alternating every 90 s), or room air (21 % O₂). Relative to basal levels of G protein activation, carbachol and DAMGO increased G protein activation by approximately 70 % across all oxygen concentrations. Compared to the room air condition, sustained hypoxia caused a significant increase in G protein activation in frontal association (FrA) region of the PFC. Region-specific comparisons revealed that intermittent and sustained hypoxia caused greater DAMGO-stimulated G protein activation in the FrA than in the PrL. The data show for the first time that hypoxia increased G protein activation in PFC. The results suggest the potential for hypoxia-induced enhancements in G protein activation to alter PFC function.

Keywords

Acetylcholine; hypoxia; neurobehavioral impairment; opioid; sleep apnea

1. Introduction

In obstructive sleep apnea (OSA) patients, upper airway occlusion can occur more than 30 times per hour and cause intermittent episodes of hypoxia (Ryan and Bradley 2005; Shamsuzzaman et al., 2003). Daytime symptoms of OSA can include impaired memory, inability to sustain attention, diminished motivation, and altered affect (Beebe and Gozal 2002). The fact that many of these neurocognitive functions are regulated, in part, by the prefrontal cortex (PFC) supports a working hypothesis that OSA disrupts PFC function (Beebe 2005; Beebe and Gozal 2002).

© 2008 Elsevier B.V. All rights reserved.

Correspondence and Proofs to: Ralph Lydic, Ph.D., Department of Anesthesiology, University of Michigan, 7433 Medical Sciences Bldg. I, 1150 W. Medical Center Drive, Ann Arbor, MI 48109-5615, VOICE: (734) 647-7831, FAX: (734) 764-9332, rlydic@umich.edu.

DISCLOSURE

None of the authors has a conflict of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The cellular and molecular mechanisms through which OSA might alter PFC function remain poorly understood. OSA is a complex syndrome involving sleep disruption, hypercapnia, and hypoxia (Beebe 2005). Functional magnetic resonance imaging studies do provide evidence of hypofrontality in OSA patients, but caution that altered PFC function cannot be solely attributed to hypoxia (Thomas et al., 2005). Such complexities have encouraged the development of rodent models aiming to help clarify the cellular and molecular effects of intermittent hypoxia on brain function (Gozal et al., 2001).

No previous studies have quantified the effect of hypoxia on G proteins in rat PFC. Therefore, the present study was designed to examine the hypothesis that the cholinergic agonist carbachol and the mu opioid agonist [D-Ala²,N-Me-Phe⁴,Gly⁵-ol] enkephalin (DAMGO) activate G proteins in the PFC and that such activation is altered by hypoxia. Rats were exposed to different levels of hypoxia and subsequently G proteins in PFC were quantified using [³⁵S]GTPγS autoradiography. Portions of these data have been presented (Hambrecht et al., 2007a).

2. Materials and methods

This study adhered to the Public Health Service policy on Humane Care and Use of Laboratory Animals (National Institutes of Health Publication 80–23). A total of 18 male Crl:CD®(SD) IGS BR (Sprague-Dawley) rats (Charles River Laboratories, Wilmington, MA), 45–50 days old, were housed under normoxic conditions in identical chambers (30 × 20 × 20 in³). Rats were maintained on a 12:12 h light-dark cycle (lights on at 6:00 a.m.). Air was set to circulate around each chamber, attached tubing, and other units at 60 L/min, resulting in one complete air turnover every 10 s. Chamber temperature was maintained at 22–24 °C. Experiments began by dividing the rats into three groups, each exposed for 14 consecutive days to one of three oxygen (O₂) conditions (Fig. 1A in vivo). Rats treated with intermittent hypoxia (n = 6) were exposed to an alternation of room air (21 % O₂) and hypoxic air (10 % O₂) every 90 s for 12 h during the light phase and room air during the dark phase. Animals in the sustained hypoxic group (n = 6) were exposed to alternating cycles of hypoxic air (10 % O₂) and hypoxic air (10 % O₂) every 90 s for 12 h during the light phase and room air during the dark phase. Control animals (n = 6) were exposed to alternating cycles of room air (21 % O₂) and room air (21 % O₂) every 90 s for 12 h during the light phase and room air during the dark phase. O₂ concentration was monitored during the entire experiment and adjusted automatically, if necessary, using custom designed, computer operated, gas valve controllers.

2.1 [³⁵S]GTPγS autoradiography

After 14 days of exposure to one of the three O₂ conditions, rats were decapitated and the brains were removed and frozen in a –30 °C bilayer of bromobutane (Fisher Scientific, Boston, MA) and isopentane (Sigma-Aldrich, St. Louis, MO). Serial coronal brain sections (20 μm thick) containing the frontal association (FrA) and prelimbic (PrL) regions (Paxinos and Watson 2005) of the PFC (Fig. 1B) were collected using a Leica CM 3050 S cryostat (Leica Microsystems, Nussloch, Germany). Brains were cut and tissue sections were collected in a specific order so that every third section belonged to one of the three in vitro treatments. This made it possible to study the effect of the different drugs on G proteins in tissue sections evenly distributed throughout the PFC. The sections were thaw-mounted onto gelatin-coated glass slides and stored at –80 °C until used in the [³⁵S]GTPγS assay.

The [³⁵S]GTPγS was purchased from Perkin Elmer Life Science (Boston, MA). Guanosine 5'-O-(γ-thio) triphosphate (GTPγS), guanosine 5'-diphosphate (GDP), the cholinergic agonist carbachol, the mu opioid agonist [D-Ala²,N-Me-Phe⁴,Gly⁵-ol] enkephalin (DAMGO), and chemicals used for buffers were obtained from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase (ADA) was purchased from Roche Applied Science (Indianapolis, IN).

The [^{35}S]GTP γ S binding assay was performed as previously described (DeMarco et al., 2003; Douglas et al., 2004; Hambrecht et al., 2007b). Slide mounted brain sections were removed from the freezer and slowly allowed to reach room temperature. Sections were then rinsed in a buffer solution (50 mM Tris-HCl, 3 mM MgCl $_2$, 0.2 mM EGTA and 100 mM NaCl, pH 7.4) for 10 min, followed by a 15 min pre-incubation in buffer solution containing 2 mM GDP and 2 U/mg ADA. For the next two hours, sections received one of the four following treatments (Fig. 1A, in vitro): To measure basal binding, tissue sections were incubated in buffer solution containing 0.04 nM [^{35}S]GTP γ S, 2 mM GDP and 2 U/mg ADA (assay buffer). To quantify carbachol-stimulated binding, incubation solution included assay buffer and 1 mM carbachol. Stimulation with DAMGO was achieved by incubation with assay buffer and 3 μM DAMGO. Previous studies have shown that 1 mM carbachol and 3 μM DAMGO are ideal for the [^{35}S]GTP γ S autoradiography (Bernard et al., 2005; Capece et al., 1998; DeMarco et al., 2003). For nonspecific binding measurements, excess unlabeled GTP γ S (10 μM) was added into the assay buffer. After incubation in one of these four assay conditions, all sections were rinsed twice in ice-cold 50 mM Tris-HCl buffer (pH 7.0) for 2 min and once in deionized ice-cold water for 30 s. Subsequently, tissue sections were dried at 4 $^{\circ}\text{C}$ under a stream of cold air before being placed in a vacuum desiccator at room temperature overnight. Autoradiography cassettes containing a Kodak BioMax MR film (Fisher Scientific, Boston, MA) were packed with tissue sections and ^{14}C microscale standards (31–883 nCi/g, Amersham Biosciences, Arlington Heights, IL). Sixteen hours after being exposed to the tissue sections and the radioactive standards, the films were developed using a Kodak X-OMAT Model 2002A film processor. In a final step, tissue sections were fixed with paraformaldehyde vapor (80 $^{\circ}\text{C}$) and stained with cresyl violet.

Exposing the slide mounted tissue sections to the receptor agonists initiated the exchange of GDP for non-hydrolyzable [^{35}S]GTP γ S, which remained bound to, and thus labeled, the receptor G protein complex. Thus, the increase in radioactivity and corresponding increase in optical density were used to quantify G protein activation while preserving brain anatomy (Laitinen 2004; Sim et al., 1997; 1995). The autoradiographic images and the corresponding cresyl violet-stained tissue sections were digitized using a Cohu CCD camera with a Nikon 60 mm objective. The digitized images were analyzed using a G3 Macintosh computer and the Scion Image 1.62c version of the NIH Image program. The FrA and PrL regions of the PFC were identified on each digitized cresyl violet section and the x-y coordinates were transferred to the corresponding autoradiographic image (Figs. 1C and D). Optical density measurements then were taken from the autoradiographic image in order to calculate total [^{35}S]GTP γ S binding. Nonspecific binding values were obtained in the same manner. Specific [^{35}S]GTP γ S binding values were calculated by subtracting mean nonspecific binding values for each PFC region from the total binding values for a given brain region. Specific [^{35}S]GTP γ S binding values were expressed as nCi/g tissue.

2.2 Statistical Analyses

For both FrA and PrL regions, the number of measurements varied with the number of sections collected along the anterior-to-posterior axis. Quantification of G protein activation was based on a total of 3,600 measures of [^{35}S]GTP γ S binding. Average binding values were calculated for each animal in an effort to avoid false-positives that can be associated with very large degrees of freedom. All binding values were calculated as 100 % of basal binding. Analysis of variance (ANOVA) for repeated measures and correlated t-tests were used because each brain contributed an average of 200 measurements. These measurements were used to determine whether G protein activation varied significantly as a function of brain region, receptor agonist, and in vivo O $_2$ treatment. A probability value of $p \leq 0.05$ was set as statistically significant.

3. Results

3.1 Cholinergic and Opioid Activation of G Proteins

The effect of carbachol on [35 S]GTP γ S binding as a function of the different oxygen treatments is plotted in Figs. 2A and B. Carbachol significantly ($p < 0.0007$) increased G protein activation over basal levels in FrA (Fig. 2A) and PrL (Fig. 2B) of rats that had been exposed to room air, intermittent hypoxia, or sustained hypoxia. In addition, G protein activation by carbachol in the FrA was significantly ($p = 0.017$) increased by sustained hypoxia compared to room air (Fig. 2A, bracket). G protein activation caused by the mu opioid agonist DAMGO as a function of in vivo treatment condition is depicted for both PFC regions in Figs. 2C and D. For all three in vivo treatment conditions, DAMGO significantly ($p < 0.0005$) increased G protein activation over basal values. Figure 2C (bracket) shows that sustained hypoxia caused significantly ($p = 0.05$) greater activation of G proteins in the FrA region than exposure to room air. ANOVA revealed no significant differences in basal binding values between room air, intermittent hypoxia and sustained hypoxia within the FrA and the PrL, respectively. The PrL showed higher basal binding values compared to the FrA and all binding values were calculated as 100 % of basal binding.

3.2 Opioid Activation of G Proteins was Region Specific

Levels of G protein activation by the mu opioid agonist DAMGO following all three oxygen treatments are summarized in Fig. 3 as a function of PFC region. DAMGO caused significantly greater G protein activation in the FrA than in the PrL following exposures to intermittent hypoxia (Fig. 3B, $p < 0.05$) or sustained hypoxia (Fig. 3C, $p < 0.01$).

Figure 4 illustrates DAMGO activated [35 S]GTP γ S binding in the FrA and PrL. These color-coded autoradiograms show that DAMGO caused greater G protein activation in the FrA than in the PrL following exposure to intermittent (Fig. 4B) or sustained (Fig. 4C) hypoxia. The individual examples of G protein activation shown in Fig. 4 are representative of the group data expressed quantitatively in Fig. 3.

4. Discussion

4.1 Hypoxia Altered Cholinergic Activation of G Proteins in PFC

Prefrontal cortical acetylcholine originates mainly from cholinergic neurons in the basal forebrain (Mesulam et al., 1983; Sarter and Bruno 2000; Semba 2000). Acetylcholine is essential for learning and memory (Hasselmo and McGaughy 2004) and rats exposed to intermittent hypoxia exhibit spatial learning deficits (Row et al., 2003) and decreased choline acetyltransferase expression in the basal forebrain (Row et al., 2007). Muscarinic cholinergic receptors are coupled to G proteins (Caulfield and Birdsall 1998) and in PFC of C57BL/6J (B6) mouse, muscarinic cholinergic autoreceptors of the M2 subtype modulate acetylcholine release (Douglas et al., 2002b). Activating postsynaptic muscarinic M1 receptors enhances excitability of the electroencephalogram recorded from PFC of B6 mouse (Douglas et al., 2002a). Microdialysis studies show increased PFC acetylcholine release in rats trained to perform an attention-demanding task (Kozak et al., 2006). Considered together, the foregoing data are consistent with the present finding that the cholinergic agonist carbachol activated G proteins throughout the PFC (Figs. 2A and B). Carbachol caused greater activation of G proteins following sustained hypoxia than following room air in FrA (Fig. 2A). In contrast, hypoxia did not alter G proteins in PrL (Fig. 2B). The [35 S]GTP γ S assay can not specify the mechanism by which sustained hypoxia increased activation of G proteins in FrA.

4.2 Hypoxia Altered Mu Opioid Activation of G Proteins within the PFC

Mu opioid receptors are widely distributed throughout the neocortex (Mansour et al., 1995) and mu opioid receptors couple exclusively to $G_{i/o}$ proteins (Birnbaumer et al., 1990). G proteins linked to mu opioid receptors can be visualized by using the [35 S]GTP γ S assay (Capece et al., 1998; Hambrecht et al., 2007b; Sim et al., 1995; Sim-Selley et al., 2000). In the FrA region, G protein activation by DAMGO varied as a function of O_2 concentration (Fig. 2C). For the PrL region (Fig. 2D) DAMGO caused no differences in G protein activation across O_2 concentrations. It is not known whether the region-specific differences in G protein activation reflect the presence of fewer mu opioid receptors in the FrA than in the PrL.

4.3 G Protein Activation Varied as a Function of PFC Region

The rat PFC is known to be functionally and anatomically heterogeneous (Gabbott et al., 2005; Morgane and Mokler 2006), and this compartmental organization provided a rationale for quantifying G protein activation in FrA and PrL regions of the PFC. These region-specific comparisons showed that DAMGO caused greater G protein activation in FrA than PrL during hypoxia (Fig. 3). Carbachol-activated [35 S]GTP γ S binding showed no significant differences between the FrA and PrL regions of the PFC (data not shown). Figure 4 provides color-coded images illustrating region-specific activation within PFC by DAMGO during intermittent and sustained hypoxia. Causes of the regional differences in G protein activation between FrA and PrL are likely to involve multiple mechanisms. For example, morphine increases $G_{\alpha i1}$ and $G_{\alpha i2}$ mRNA levels in PFC, which could increase the pool of available G proteins (Kaewsuk et al., 2001). Alternatively, the FrA may possess more opioid receptors than the PrL. During hypoxia, transcription is regulated by the hypoxia inducible factor-1 (HIF-1) (Rocha 2007), and PrL and FrA regions of PFC may differ in HIF-1 expression and regulatory components. The PFC is also neurochemically heterogeneous (Robbins 2000; 2005) and the present finding of regional differences in G protein activation encourages future studies using additional agonists in order to characterize the effects of hypoxia on other G protein coupled receptors within the PFC.

4.4 Study Limitations

The strengths and limitations of the [35 S]GTP γ S assay have been reviewed elsewhere (Bernard et al., 2005; Harrison and Traynor 2003; Laitinen 2004; Sim et al., 1995). Briefly, limitations include the fact that although the [35 S]GTP γ S assay preferentially labels inhibitory G proteins, specifying the G protein subtypes activated by the different ligands is not possible with the [35 S]GTP γ S assay. Additionally, no conclusions can be drawn about pre- versus postsynaptic location of activated G proteins. Relative to the room air condition, brains that were exposed to sustained hypoxia (bracket in Figs. 2A and C) showed statistically significant increases in G protein activation by carbachol and DAMGO. Region-specific analyses revealed greater G protein activation by DAMGO in FrA than PrL during intermittent (Figs. 3B) and sustained (Fig. 3C) hypoxia. How these small but statistically significant regional differences are reflected in prefrontal cortical function cannot be addressed by the [35 S]GTP γ S assay. It is known, however, that cellular changes of small magnitude can significantly alter brain function. For example, 15 % alteration in acetylcholine release in prefrontal cortex causes significant activation of the cortical electroencephalogram (Douglas et al., 2002a). G proteins function as a complex network that amplifies transmembrane signaling. Thus, a small change in G protein activation can significantly alter distal signal transduction cascades (Laitinen 2004; Tanase et al., 2001).

4.5 Functional Considerations and Conclusions

The prefrontal cortex regulates executive functions such as working memory, speed of information processing, and focused attention (Marklund et al., 2007). In rodent, homologs to

these executive functions can be inferred from performance on the Morris water maze. Previous studies showed that episodic, but not sustained, hypoxia caused decreased Morris maze performance and increased apoptosis within CA1 hippocampus and unspecified cortical areas ranging from bregma 2.3 to 4.8 mm (Gozal et al., 2001). The good parallel between rodent and human data suggests that hypoxia caused by sleep apnea contributes to deficits in prefrontal cortical function (Beebe 2005; Beebe and Gozal 2002). In the present study sustained hypoxia enhanced G protein activation in FrA (Figs. 2A and C). Other studies show that sustained hypoxia experienced at extremely high altitude causes synaptic disruption and neurodegeneration in prefrontal cortex (Maiti et al., 2008). In contrast to the previous finding that episodic hypoxia caused neurodegeneration (Gozal et al., 2001), the present results show that intermittent hypoxia caused region specific enhancement of G protein activation (Fig. 3B). The degree to which these differences are a function of duration of hypoxia, different neurochemical markers, and different brain regions remains unclear. Additionally, the increase in G proteins may reflect a compensatory response to hypoxia. Others have reported that over time there is a decrease in the extent of neurodegeneration associated with the sustained hypoxia of altitude (Maiti et al., 2008).

The finding that the mu opioid agonist DAMGO caused region specific changes in PFC G proteins (Figs. 3 and 4) also has functional implications. The PFC interacts with the brainstem respiratory network (Pattinson et al., 2009) and opioid therapy can worsen hypoxia severity (Farney et al., 2003). Additionally, opioids can cause delirium by disrupting neurotransmission in the PFC (Osman et al., 2005). The present results encourage future studies to elucidate the mechanisms by which opioids and hypoxia alter G proteins in PFC.

In conclusion, the present finding that hypoxia enhances activation of G proteins in PFC suggests for the first time the potential for hypoxia-induced G proteins to alter PFC function. These results provide cellular-level evidence supporting the hypothesis that the neurobehavioral deficits associated with hypoxia may result, in part, from alterations in the PFC (Beebe 2005; Beebe and Gozal 2002).

Acknowledgments

We thank D. Ignasiak, S. Jiang, and M. A. Norat for expert assistance. For statistical input we thank K. Welch and the University of Michigan Center for Statistical Consultation and Research.

GRANTS: National Institutes of Health (HL40881, HL57120, HL65272, MH45361, HL69932, and 2P50HL60296).

References

- Beebe DW. Neurobehavioral effects of obstructive sleep apnea: an overview and heuristic model. *Curr. Opin. Pulm. Med* 2005;11:494–500. [PubMed: 16217174]
- Beebe DW, Gozal D. Obstructive sleep apnea and the prefrontal cortex: towards a comprehensive model linking nocturnal upper airway obstruction to daytime cognitive and behavioral deficits. *J. Sleep Res* 2002;11:1–16. [PubMed: 11869421]
- Bernard, R.; Lydic, R.; Baghdoyan, HA. Hypocretin receptor-activated G proteins revealed by [³⁵S] GTPγS autoradiography. In: Nishino, S.; Sakurai, T., editors. *The Orexin/Hypocretin System: Physiology and Pathophysiology*. Humana Press Inc.; Totowa, NJ: 2005. p. 83-96.
- Birnbaumer L, Abramowitz J, Brown AM. Receptor-effector coupling by G proteins. *Biochim. Biophys. Acta* 1990;1031:163–224. [PubMed: 2160274]
- Capece ML, Baghdoyan HA, Lydic R. Opioids activate G proteins in REM sleep-related brain stem nuclei of rat. *Neuroreport* 1998;9:3025–3028. [PubMed: 9804309]
- Caulfield MP, Birdsall NJM. International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev* 1998;50:279–290. [PubMed: 9647869]

- DeMarco GJ, Baghdoyan HA, Lydic R. Differential cholinergic activation of G proteins in rat and mouse brainstem: relevance for sleep and nociception. *J. Comp. Neurol* 2003;457:175–184. [PubMed: 12541317]
- Douglas CL, Baghdoyan HA, Lydic R. Postsynaptic muscarinic M1 receptors activate prefrontal cortical EEG of C57BL/6J mouse. *J. Neurophysiol* 2002a;88:3003–3009. [PubMed: 12466425]
- Douglas CL, Baghdoyan HA, Lydic R. Prefrontal cortex acetylcholine release, EEG slow waves, and spindles are modulated by M2 autoreceptors in C57BL/6J mouse. *J. Neurophysiol* 2002b;87:2817–2822. [PubMed: 12037184]
- Douglas CL, DeMarco GJ, Baghdoyan HA, Lydic R. Pontine and basal forebrain cholinergic interaction: implications for sleep and breathing. *Resp. Physiol. Neurobiol* 2004;143:251–262.
- Farney RJ, Walker JM, Cloward TV, Rhondeau S. Sleep-disordered breathing associated with long-term opioid therapy. *Chest* 2003;123:632–639. [PubMed: 12576394]
- Gabbott PLA, Warner TA, Jays PRL, Salway P, Busby SJ. Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. *J. Comp. Neurol* 2005;492:145–177. [PubMed: 16196030]
- Gozal D, Daniel JM, Dohanich GP. Behavioral and anatomical correlates of chronic episodic hypoxia during sleep in the rat. *J. Neurosci* 2001;21:2442–2450. [PubMed: 11264318]
- Hambrecht VS, Vlisides PE, Row BW, Gozal D, Baghdoyan HA, Lydic R. G proteins in the prefrontal cortex (PFC) of Sprague-Dawley rat are differentially activated as a function of oxygen (O₂) status and PFC region. *Sleep* 2007a;30(Abstract), 0019
- Hambrecht VS, Vlisides PE, Row BW, Gozal D, Baghdoyan HA, Lydic R. Hypoxia modulates cholinergic but not opioid activation of G proteins in rat hippocampus. *Hippocampus* 2007b;17:934–942. [PubMed: 17598161]
- Harrison C, Traynor JR. The [³⁵S]GTPγS binding assay: approaches and applications in pharmacology. *Life Sci* 2003;74:489–508. [PubMed: 14609727]
- Hasselmo ME, McGaughy J. High acetylcholine levels set circuit dynamics for attention and encoding and low acetylcholine levels set dynamics for consolidation. *Prog. Brain Res* 2004;145:207–231. [PubMed: 14650918]
- Kaewsuk S, Hutamekalin P, Kettermann AJ, Khotchabhakdi N, Govitrapong P, Casalotti SO. Morphine induces short-lived changes in G-protein gene expression in rat prefrontal cortex. *Eur. J. Pharmacol* 2001;411:11–16. [PubMed: 11137853]
- Kozak R, Bruno JP, Sarter M. Augmented prefrontal acetylcholine release during challenged attentional performance. *Cereb. Cortex* 2006;16:9–17. [PubMed: 15788700]
- Laitinen JT. [³⁵S]GTPγS autoradiography: A powerful functional approach with expanding potential for neuropharmacological studies on receptors coupled to Gi family of G proteins. *Curr. Neuropharmacol* 2004;2:191–206.
- Maiti P, Singh SB, Mallick B, Muthuraju S, Ilavazhagan G. High altitude memory impairment is due to neuronal apoptosis in hippocampus, cortex and striatum. *J Chem Anat* 2008;36:227–238.
- Mansour A, Fox CA, Akil H, Watson SJ. Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. *Trends Neurosci* 1995;18:22–29. [PubMed: 7535487]
- Marklund P, Fransson P, Cabeza R, Heterosson KM, Ingvar M, Nyberg L. Sustained and transient neural modulations in prefrontal cortex related to declarative long-term memory, working memory, and attention. *Cortex* 2007;44:22–37. [PubMed: 17334205]
- Mesulam MM, Mufson EJ, Levey AI, Wainer BH. Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey. *J. Comp. Neurol* 1983;214:170–197. [PubMed: 6841683]
- Morgane PJ, Mokler DJ. The limbic brain: Continuing resolution. *Neurosci. Biobehav. Rev* 2006;30:119–125. [PubMed: 16115685]
- Osman NI, Baghdoyan HA, Lydic R. Morphine inhibits acetylcholine release in rat prefrontal cortex when delivered systemically or by microdialysis to basal forebrain. *Anesthesiology* 2005;103:779–787. [PubMed: 16192770]

- Pattinson KTS, Mitsis GD, Harvey AK, Jbabdi S, Dirckx S, Mayhew SD, Rogers R, Tracey I, Wise RG. Determination of the human brainstem respiratory control network and its cortical connections in vivo using functional and structural imaging. *NeuroImage* 2009;44:295–305. [PubMed: 18926913]
- Paxinos, G.; Watson, C. *The Rat Brain In Stereotaxic Coordinates*. Academic Press; San Diego, CA: 2005.
- Robbins TW. Chemical neuromodulation of frontal-executive functions in humans and other animals. *Exp. Brain Res* 2000;133:130–138. [PubMed: 10933217]
- Robbins TW. Chemistry of the mind: neurochemical modulation of prefrontal cortical function. *J. Comp. Neurol* 2005;493:140–146. [PubMed: 16254988]
- Rocha S. Gene regulation under low oxygen: holding your breath for transcription. *Trends Biochem. Sci* 2007;32:389–397. [PubMed: 17624786]
- Row BW, Kheirandish L, Cheng Y, Rowell PP, Gozal D. Impaired spacial working memory and altered choline acetyltransferase (CHAT) immunoreactivity and nicotinic receptor binding in rats exposed to intermittent hypoxia during sleep. *Behav. Brain Res* 2007;177:308–314. [PubMed: 17218023]
- Row BW, Liu R, Xu W, Kheirandish L, Gozal D. Intermittent hypoxia is associated with oxidative stress and spatial learning deficits in the rat. *Am. J. Respir. Crit. Care Med* 2003;167:1548–1553. [PubMed: 12615622]
- Ryan CM, Bradley TD. Pathogenesis of obstructive sleep apnea. *J. Appl. Physiol* 2005;99:2440–2450. [PubMed: 16288102]
- Sarter M, Bruno JP. Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: differential afferent regulation of the basal forebrain by telencephalic and brainstem afferents. *Neuroscience* 2000;95:933–952. [PubMed: 10682701]
- Semba K. Multiple output pathways of the basal forebrain: organization, chemical heterogeneity, and roles in vigilance. *Behav. Brain Res* 2000;115:117–141. [PubMed: 11000416]
- Shamsuzzaman ASM, Gersh BJ, Somers VK. Obstructive sleep apnea: implications for cardiac and vascular disease. *J.A.M.A* 2003;290:1906–1914.
- Sim LJ, Selley DE, Childers SR. Autoradiographic visualization in brain of receptor-G protein coupling using [³⁵S]GTPγS binding. *Methods Mol. Biol* 1997;83:117–132. [PubMed: 9210141]
- Sim LJ, Selley DE, Childers SR. In vitro autoradiography of receptor-activated G proteins in rat brain by agonist-stimulated guanylyl 5'-[γ-³⁵S]thio]-triphosphate binding. *Proc. Natl. Acad. Sci. U.S.A* 1995;92:7242–7246. [PubMed: 7638174]
- Sim-Selley LJ, Selley DE, Vogt LJ, Childers SR, Martin TJ. Chronic heroin self-administration desensitizes μ opioid receptor-activated G-proteins in specific regions of rat brain. *J. Neurosci* 2000;20:4555–4562. [PubMed: 10844025]
- Tanase D, Martin WA, Baghdoyan HA, Lydic R. G protein activation in rat ponto-mesencephalic nuclei is enhanced by combined treatment with a mu opioid and an adenosine A₁ receptor agonist. *Sleep* 2001;24:52–62. [PubMed: 11204054]
- Thomas RJ, Rosen BR, Stern CE, Weiss JW, Kwong KK. Functional imaging of working memory in obstructive sleep-disordered breathing. *J. Appl. Physiol* 2005;98:2226–2234. [PubMed: 15677733]

Fig 1.

Schematic of study design and techniques used to quantify G protein activation in the frontal association cortex (FrA) and prelimbic cortex (PrL) regions of the prefrontal cortex (PFC).

A. Outline of the in vivo and in vitro phases of this study. During the in vivo phase, rats were exposed for 14 days to either room air, intermittent hypoxia, or sustained hypoxia.

Subsequently, rats were decapitated and brains were removed and immediately frozen. For the following in vitro phase, brains were sectioned serially and assayed for G protein activation caused by the cholinergic receptor agonist carbachol and the mu opioid receptor agonist DAMGO. The number of animals (n) is shown for each in vivo treatment group. **B.** Schematic showing a sagittal view of the rat brain (Paxinos and Watson 2005) indicating the rostral to caudal extension from the beginning of the FrA to the end of the PrL (area indicated by vertical lines and double headed arrow). **C** and **D.** Schematic of the technique for obtaining region-

specific measurements of [^{35}S]GTP γ S binding in PFC regions. Circles show where PFC regions were localized on digitized cresyl violet-stained images (C). The average measurement area for the FrA region was 80 square pixels and for the PrL region 40 square pixels. The x and y coordinates of these circles were transferred onto the corresponding [^{35}S]GTP γ S autoradiographic images (D). Optical density values taken from the autoradiographic images were proportional to G protein activation quantified by the amount of [^{35}S]GTP γ S binding.

Fig 2.

G protein activation in the frontal association cortex (FrA) and prelimbic cortex (PrL) as a function of exposure to room air (RA), intermittent hypoxia (IH), and sustained hypoxia (SH).

A. Carbachol significantly (*) increased G protein activation in the FrA compared to basal (control) values for all three in vivo treatments. In addition, G protein activation by carbachol was significantly (†, bracket) increased by sustained hypoxia when compared with room air.

B. Relative to basal values, carbachol significantly (*) increased $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding in the PrL for all oxygen conditions. **C.** DAMGO significantly (*) increased G protein activation in the FrA compared to basal levels for room air, intermittent hypoxia, and sustained hypoxia. Furthermore, rats exposed to sustained hypoxia revealed a significant (†, bracket) increase in $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding compared to room air treated animals.

D. In the PrL, DAMGO significantly (*) increased G protein activation compared to basal levels following exposure to room air, intermittent hypoxia, and sustained hypoxia.

Fig 3.

DAMGO-stimulated G protein activation as a function of prefrontal cortex regions for each in vivo oxygen condition. **A.** Rats exposed to room air (RA) displayed no significant difference in DAMGO-activated [³⁵S]GTPγS binding levels between FrA and PrL. **B.** Animals exposed to intermittent hypoxia (IH) revealed significantly (*) greater G protein activation in the FrA than in the PrL. **C.** DAMGO also significantly (*) increased G protein activation in the FrA for rats treated with sustained hypoxia (SH).

Fig 4.

Region-specific activation of G proteins in prefrontal cortex by DAMGO and hypoxia. **A.** Coronal atlas plates (Paxinos and Watson 2005) show the frontal association cortex (FrA; left) and prelimbic cortex (PrL; right) regions of the prefrontal cortex (PFC). The red boxes localize the areas enlarged below. **B.** Color-coded autoradiogram of the FrA (left) and PrL (right). Color scale at bottom indicates lower levels of G protein activation correspond to blue and higher levels of G protein activation correspond to red. Compared to the PrL, DAMGO-activated [³⁵S]GTPγS binding in the FrA was increased after exposure to intermittent hypoxia. **C.** After exposure to sustained hypoxia, DAMGO also increased G protein activation in the FrA compared to the PrL. Dotted lines in the color figures (B and C) show the area within FrA and PrL where measurements were obtained.