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Rhythmic oxygen levels reset circadian clocks through HIF1α

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Running title: Oxygen as a resetting cue for circadian clocks

Abstract

The mammalian circadian system consists of a master clock in the brain that synchronizes subsidiary oscillators in peripheral tissues. The master clock maintains phase coherence in peripheral cells through systemic cues such as feeding-fasting and temperature cycles. Here we examined the role of oxygen as a resetting cue for circadian clocks. We continuously measured oxygen levels in living animals and detected daily rhythms in tissue oxygenation. Oxygen cycles, within the physiological range, were sufficient to synchronize cellular clocks in HIF1α-dependent manner. Furthermore, several clock genes responded to changes in oxygen levels through HIF1α. Finally, we found that a moderate reduction in oxygen levels for a short period accelerates the adaptation of wild type but not of HIF1α-deficient mice to the new time in a jet lag protocol. We conclude that oxygen, via HIF1α activation, is a resetting cue for circadian clocks and propose oxygen modulation as therapy for jet lag.

Introduction

In mammals, a master circadian clock in the brain synchronizes subsidiary oscillators in peripheral tissues. The presence of cell autonomous oscillators in almost every cell in the body raises the question how these oscillators are temporally coordinated. Because these cellular oscillators anticipate and function together in a proactive manner to environmental changes their temporal synchronization is critical (Schibler et al., 2015).

The molecular makeup for circadian rhythm generation is based on interlocked negative transcription-translation feedback loops (Feng and Lazar, 2012; Partch et al., 2014). The basic helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) proteins BMAL1 and CLOCK heterodimerize and drive the expression of the *Period* (i.e., *Per1, Per2* and *Per3*) and *Cryptochrome* (i.e., *Cry1* and *Cry2*) genes. Subsequently, PER and CRY proteins accumulate and repress the transcription of their own genes. An auxiliary essential feedback loop includes the orphan nuclear receptors of the REV-ERB and ROR families.

The quest for universal timing cues for peripheral clocks in mammals has yielded two principal entrainment signals: feeding and temperature. Both feeding rhythms and temperature cycles were shown to synchronize peripheral clocks and even uncouple them from the master clock in the brain (e.g., daytime restricted feeding), (Brown et al., 2002; Buhr et al., 2010; Damiola et al., 2000; Saini et al., 2012; Stokkan et al., 2001; Vollmers et al., 2009). Notably, both nutrient ingestion/processing and maintenance of body temperature are tightly linked to oxygen consumption. Furthermore, oxygen is readily available and recognized by most cells of the body, rendering it as an ideal signaling molecule. Hence, we posited that oxygen might function as a systemic timing cue for circadian clocks, a conjuncture that so far was never tested. For oxygen to function as a resetting cue for circadian oscillators two requirements would be necessary: (i) it should exhibit daily rhythms and (ii) peripheral oscillators must be sensitive to variation in oxygen levels within the physiological range.

We tested this hypothesis and identified daily rhythms in blood and tissue oxygen levels. These physiological oxygen rhythms were sufficient to synchronize clocks in cultured cells in HIF1α-dependent manner. Furthermore, we found that moderate reduction in oxygen ambient levels, even for a short period as 2 h, accelerates the adaptation of wild type but not of HIF1α-deficient mice to the new time in a jet lag protocol. We suggest that oxygen functions as a prominent resetting cue for circadian clocks through HIF1α activation and propose oxygen modulation as a supportive therapy for jet lag.

Results and Discussion

Daily rhythms in blood and tissue oxygen levels

To test whether there are daily variations in oxygen levels we first monitored the oxygen consumption rate of mice using metabolic cages (Figure 1A). Oxygen consumption rate was elevated in the dark phase compared to the light phase. The increase in oxygen consumption during the night in mice coincided with their activity onset and food ingestion, both of which consume oxygen. This result incited us to examine whether there are daily changes in blood and tissue oxygen levels as well. We measured the oxygen levels in blood from mice using an oxygen optical fiber and found that they oscillate with zenith levels during the dark phase (Figure 1B). Concurrently, we employed a telemetric oxygen electrode device to continuously monitor oxygen levels in kidney of freely moving rats for several consecutive days (Figure 1C). Kidney oxygenation was rhythmic, and reached its peak levels during the dark phase (Figure 1D and 1E). In line with previous reports, the mean daily O_2 levels in kidney were ~7% (6.94 ± 1.34%, mean ± SEM, n=5), (Carreau et al., 2011). The range of the rhythmic daily changes in kidney oxygenation was ~3% O_2 (Figure 1F). Taken together, our analyses identified daily oscillations in oxygen consumption and oxygen levels in blood and kidney with peak levels during the night.

The Hypoxia-Inducible Factor 1α (HIF1α) is a bHLH-PAS domain-containing transcription factor that responds to and participates in oxygen homeostasis (Majmundar et al., 2010). Under normoxia HIF1α is rapidly degraded via the Von Hippel-Lindau (VHL)-mediated ubiquitin-proteasome degradation pathway; however, once oxygen levels decrease, HIF1α degradation is inhibited and HIF1α accumulates. Hence, HIF1α protein levels are tightly regulated post-transcriptionally and inversely correspond to oxygen levels. Of note, HIF1α was previously reported to heterodimerize with other bHLH-PAS domain-containing proteins such as BMAL1 (Hogenesch et al., 1998). The above-described rhythms in oxygen

levels prompted us to examine whether they incite daily changes in HIF1α levels. While *Hif1α* transcript levels were relatively constant throughout the day (Figure S1A), HIF1α nuclear protein levels exhibited daily rhythms with peak levels at Zeitgeber Time (ZT) ~8 and ~12 in mouse kidney (Figures 1G and S1B) and brain (Figures 1H and S1C) respectively. Thus, the accumulation of HIF1α in brain is ~4 h delayed compared to kidney, and corresponds to the peak in REV-ERBα protein levels in both tissues.

Overall, we uncovered daily rhythms in blood and tissue oxygenation together with daily oscillations in HIF1α protein levels.

Physiological oxygen rhythms synchronize circadian clocks in cultured cells in HIF1α-dependent manner

We set out next to examine whether physiological rhythms in oxygen levels, namely 5% and 8% O_2 nadir and zenith respectively, can synchronize circadian clocks in a population of cultured cells. We made special efforts to minimize any potential confounding effects by: (i) refraining from using firefly luciferase based circadian reporters, as the luciferase enzymatic activity is sensitive to oxygen levels (Doran et al., 2011), (ii) maintaining the incubator gas composition and temperature as constant as possible. To this aim we used special chambers with CO_2, O_2 and temperature controls (see experimental procedures), (Figure 2A). Temperature, CO_2, and O_2 levels were continuously monitored throughout the experiment with constant temperature of 37ºC, 5% CO_2 and O_2 levels as indicated. To generate rhythms in oxygen levels O_2 was replaced with the inherent gas nitrogen. We applied the following experimental scheme; 24 h after cells (i.e., Hepa-1c1c7 or NIH3T3) were seeded, they were exposed to 3 consecutive cycles of 12 h 5%, 12 h 8% O_2 each (mimicking physiological O_2 rhythms), and subsequently released to constant 8% O_2 (free running). The control cells were maintained under constant 8% O_2 throughout the entire experiment (Figure 2B). Samples were

collected at 4 h intervals in the course of the free running period (Day 4) and the expression level of clock genes was determined. Due to the weak coupling between cells in culture, clocks in individual cells fail to maintain phase coherence with their neighboring cells after several days (Nagoshi et al., 2004). Thus, as expected, the transcript levels of the various clock genes were relatively constant throughout the circadian cycle in a cell population cultured under constant 8% O_2 for 4 successive days. By contrast, cells that were exposed to physiological oxygen rhythms exhibited rhythmic expression of clock genes (Hepa-1c1c7 and NIH3T3 cells, Figure 2C and Figure S2A respectively). Importantly, the phase relation, namely the relative peak time expression of the different clock genes, highly resembled the one obtained with Dexamethasone pulse that is widely used to synchronize clocks in culture (Figure S2B) and in mice (Figure S1A). Our results indicated that physiological oxygen rhythms reset the molecular clock in cultured cells.

HIF1α not only responds to changes in oxygen levels but also plays a critical role in oxygen homeostasis through gene expression regulation (Majmundar et al., 2010). We therefore examined the role of HIF1α in resetting the molecular clock upon oxygen rhythms. To this end, we employed *Hif1α* siRNA to specifically knockdown *Hif1α* (*Hif2α* transcript levels were not affected by knockdown of *Hif1α*). Remarkably, oxygen rhythms failed to elicit cyclic expression of clock genes in *Hif1α* deficient cells, and the expression levels of *Rev-erbα, Rorα, Per1, Per2, Cry1, Cry2* and *Dbp* were constant low compared to control cells (Figure 2D). We noticed a prominent effect for *Hif1α* knockdown on *Cry2* and *Rorα* expression, in particular, as their transcript levels were substantially lower in *Hif1α* deficient cells already at CT0. Analysis of protein levels of several clock genes corroborated the gene expression data (Figure 2E). Notably, CLOCK protein levels were elevated in *Hif1α* deficient cells.

Thus, in *Hif1α* deficient cells we failed to detect rhythmic expression of clock genes. This raised the possibility that HIF1α is either absolutely required for circadian rhythmicity, namely a core clock component, or specifically essential for clock resetting by oxygen rhythms. To distinguish between these two scenarios, we examined the requirement of HIF1α for resetting the clock by Dexametahsone. Knockdown of the *Clock* gene, a principal component of the core clock circuitry, completely abolished circadian rhythmicity (Figure S2B). By contrast *Hif1α* knockdown had little effect on the rhythmic expression of clock genes in cells synchronized by Dexamethasone (Figures 2F). The expression profiles of *Clock, Bmal1, Rev-erbα, Cry1* and *Dbp* were very similar irrespectively of *Hif1α* levels, and the rhythmicity of *Rorα, Per1, Per2,* and *Cry2* was preserved yet their expression levels were lower.

Taken together, our results evinced that HIF1α is specifically required for resetting the molecular clock by oxygen rhythms and not by Dexamethasone, suggesting that HIF1α is not an integral component of the core clock circuitry but rather functions upstream to the clock in response to changes in oxygen levels. We therefore concluded that HIF1α is the molecular link between oxygen and the circadian clock.

The effect of oxygen levels on core clock gene expression

We sought to identify potential downstream effector/s within the core clock circuitry that connect oxygen rhythms and HIF1α with circadian clock resetting. First, we examined the expression levels of clock genes in cultured cells throughout an oxygen cycle (Cue), namely 12 h of 5% O_2 followed by 12 h of 8% O_2 (Figure 3A). The transcript levels of *Rev-erbα, Rorα, Per1, Per2* and *Cry2* were up regulated in response to decrease in oxygen levels to 5% (Figure 3B). *Cry1* levels were only induced once oxygen levels were restored to 8%, whereas the expression levels of *Clock* and *Bmal1* were mostly unaffected. Next we examined whether

HIF1α is required for the induction of clock genes in the course of an oxygen cycle. We found that the induction of *Cry2*, *Rorα*, *Per2, Cry1,* and as expected *Glut1*, a known target of HIF1α (Chen et al., 2001), was blunted upon knockdown of *Hif1α* (Figure 3C). Remarkably, the transcript levels of both *Cry2* and *Rorα* were down regulated in the absence of HIF1α already under 8% O_2 (i.e., ZT0), (Figure 3C), and responded to decrease in oxygen levels in a dose-dependent and HIF1α−dependent manner (Figure S3A). Moreover, their transcript levels were sensitive to variations in oxygen levels within the physiological range, namely 3% O_2, (Figure S3B). In comparison to different known HIF1α target genes, such as *Glut1*, *Pdk1* and *Ldha*, both *Cry2* and *Rorα* appeared to be highly sensitive to small changes in O_2 levels, similar to *Glut1* (Figure S3A and S3B).

Bioinformatics analysis of the *Cry2* gene revealed several Hypoxia Response Element (HRE) and E-box motifs (Figure S3E); these elements are pertinent for HIF1α and BMAL1 binding respectively. In line with previous genome wide Chromatin Immunoprecipitation (ChIP) studies (Koike et al., 2012; Rey et al., 2011), our ChIP experiments showed rhythmic binding of BMAL1 specifically to a region within the *Cry2* promoter that contains both E-box and HRE motifs (i.e., block D), with peak binding at ZT8 (Figure S3F). Likewise, we managed to ChIP BMAL1 specifically on this region in chromatin prepared from mouse kidney and cultured cells (Figure S3G, S3H and S3I). The presence of HRE prompted us to test the binding of HIF1α to the *Cry2* gene, however we failed to detect any specific binding (Figure S3F and S3G). It might be that HIF1α indeed binds to the *Cry2* gene but we could not identify it either due to technical reasons or since it binds to other *Cry2* genomic regions. Remarkably, although hypoxia induced the expression of *Cry2*, it did not affect the binding of BMAL1 to the *Cry2* promoter (Figure S3H and S3I).

We reasoned that both *Cry2* and *Rorα* are potential candidates that connect oxygen-HIF1α axis and the circadian clock, as they readily responded to changes in oxygen levels in

HIF1α–dependent manner. To examine their potential role as the molecular link between oxygen-HIF1α axis and the circadian clock, we tested whether they can phenocopy the effect of HIF1α knockdown on circadian clock resetting by oxygen rhythms. Knockdown of *Cry2* primarily resulted in phase delay in the expression of clock genes in response to both oxygen rhythms and Dexamethasone (Figure 3D and S3C respectively). While knockdown of *Rorα* had some effect on the daily expression levels of several clock genes following oxygen rhythms and very little effect upon Dexamethasone (Figure 3E and S3D respectively). Yet, although both responded to changes in oxygen levels in HIF1α-dependent manner, neither knockdown of *Cry2* nor of *Rorα* mimicked the effect of *Hif1α* knockdown. It is conceivable that resetting the circadian clock by the oxygen-HIF1α axis is mediated through the concerted action of several clock genes and potentially other factors yet to be identified.

Low oxygen pulse accelerates the adaptation of mice in a jet lag protocol

To examine the relevance of the above findings in the context of the whole animal, we examined the effect of moderate reduction in oxygen levels on the daily voluntary locomotor activity of mice under different light-dark regimens. Upon 12 h light-dark cycles mice exhibit robust rest-activity cycles, with activity onset every 24 h once light is turned off. This activity pattern is preserved in mice under constant dark, albeit with a slightly shorter period than 24 h (Partch et al., 2014). We first examined whether cycles of 12 h of 21% and 12 h of 16% O_2 in the subjective dark and light phase respectively, will maintain animals on a precise 24 h schedule in constant dark. The circadian period however was similar between mice that were housed under constant versus rhythmic oxygen levels (Figure S4A and S4B).

   One of the key properties of the circadian clock is its phase resetting to a new lighting schedule. We therefore tested the effect of ambient oxygen levels on an experimental jet lag protocol of 6 h phase advance in the lighting schedule. We found that 12 h of 16% O_2 prior to

the shift considerably accelerate the adaptation of mice to the new lighting schedule (Figure 4A and 4B). Even a short pulse of 2 h of 14% O_2 following the shift in the lighting schedule was sufficient to shorten the adaptation time (Figure 4C and 4D). Next, we examined whether this effect, similar to the effect in cultured cells, is HIF1α-dependent. Since *Hif1α* null homozygous mice are nonviable (Iyer et al., 1998), we tested *Hif1α* heterozygous mice. *Hif1α* transcript and protein levels were decreased by ~50% in *Hif1α*$^{+/-}$ compared to their wild type littermates (Figure S4C and S4D). *Hif1α* deficient mice did no differ from their wild type littermates in their circadian period (Figure S4E and S4F) or in their adjustment to 6 h shift in the lighting schedule (Figure 4E and 4F). However, in contrast to wild type mice, neither 12 h of 16% O_2, nor 2 h of 14% O_2, accelerated the adaptation of HIF1α deficient mice to the new time, upon jet lag protocol (Figure 4G-4J), suggesting that HIF1α is required for oxygen resetting of circadian clocks in mice as well. In this conjuncture, we analyzed the expression levels of clock genes in whole brain samples in response to 2 h of 14% O_2 for wild type and *Hif1α* deficient littermates (Figure S4G). We found a small but statistical significant induction of *Cry2* similar to *Ldha*, an HIF1α target, that was abrogated in *Hif1α* deficient mice. Notably, both *Per1* and *Cry1* transcript levels were down regulated in *Hif1α* deficient mice irrespectively of oxygen levels.

We conclude that oxygen functions as a prominent resetting cue for circadian clocks in HIF1α-dependent manner (Figure 4I) and propose oxygen modulation as a supportive therapy for jet lag. Notably, the DNA binding activity of NPAS2-BMAL1 heterodimers is regulated by carbon monoxide (Dioum et al., 2002). Thus, different gases (i.e., oxygen, carbon monoxide) appear to play a role in circadian clock control. It is plausible that impaired tissue oxygenation under pathological conditions such as cardiovascular diseases, might also affect the clock function, and consequently contribute to the pathophysiology of the disease. On a different note, in the majority of modern commuters airplanes, the cabin oxygen pressure corresponds to

~16% O_2 (Cottrell, 1988) and the aviation industry is investing substantial funds and efforts to improve and increase the cabin oxygen levels to 21% O_2. This should be reconsidered in view of the beneficial effect of reduced oxygen levels in jet lag recovery that are reported here.

Experimental Procedures

Cell Culture, and Reagents

Hepa-1c1c7 and NIH3T3 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 100 units/ml penicillin, 100 mg/ml streptomycin and cultured at 37°C in a humidified incubator with 5% CO_2. SMARTpool ON-TARGET plus mouse *Hif1α* (L-040638), *Cry2* (L-040486), *Rorα* (L-040430), and control siRNA (D-001810), (Dharmacon) were delivered into the cells with Lipofectamine RNAi reagent 48 h prior to the experimental procedure. Cells were synchronized with 100 nM Dexamethasone (Sigma) treatment for 20 min. Experiments with different oxygen levels were conducted in special chambers with O_2, CO_2 and temperature control (Coy Laboratory).

Oxygen measurements

The oxygen consumption rate of mice was monitored using the Phenomaster metabolic cages (TSE Systems). Blood oxygen levels were measured with FireStingO$_2$ oxygen meter, Pyroscience©. Telemetry-based recordings of renal pO_2 were performed as previously described (Koeners et al., 2013; Koeners et al., 2016) and detailed in supplemental information.

RNA Preparation and Real-Time PCR analysis

RNA extraction and transcript quantification by real-time PCR were carried out as previously described (Adamovich et al., 2014). Synthesis of cDNA was done using qScript™ cDNA SuperMix (Quanta Biosciences). Real-time PCR measurements were performed using SYBR green or Taqman probes with LightCycler II machine (Roche) and normalized to the geometrical mean of 3 housekeeping genes: *Tbp*, *Hprt*, and *Rplp0*. Primers and probes are listed under Supplementary experimental procedure.

Protein extraction, Gel electrophoresis and Immunoblotting

Mouse kidney nuclei and brain were isolated as previously described (Aviram et al., 2016). Nuclei and cultured cells were homogenized in RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% Na-deoxycholate, 0.1% SDS, 50 mM Tris-Hcl pH 8, 1 mM dithiothreitol) supplemented with protease inhibitors (1 mM N-(α-aminoethyl) benzene-sulfonyl fluoride, 40 µM bestatin, 15 µM E65, 20 µM leupeptin, 15 µM pepstatin; (Sigma)). The extracts were centrifuged to remove cell debris at 13,000 rpm for 10 min at 4°C. Samples were heated at 95°C for 5 min in Laemmli sample buffer and analyzed by SDS-PAGE and immunoblot. Antibodies used: rabbit anti CLOCK, CRY2, PER2, and REV-ERBα (Asher et al., 2010), Mouse anti HIF1α (Santa Cruz 3C144), TUBULIN and U2AF (Sigma).

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Author Contributions

G.A. and Y.A wrote the paper. G.A., Y.A., and B.L., designed experiments and conducted them together with M.G. and M.P.K.

Figure legends

Figure 1. Daily rhythms in blood and tissue oxygen levels. A., Oxygen Consumption Rate (OCR) was measured using metabolic cages for 3 consecutive days. Data are presented as the mean of 8 mice (left panel). The mean ± SD OCR levels during the light and dark phase are presented in bar graph (right panel). **B.,** The blood oxygen levels were measured using an oxygen optical fiber. Data are presented as mean ± SD, with 4 animals per time point. **C.,** A schematic illustration of an oxygen telemetric measuring device for continuous measurements of oxygen levels in kidney of freely moving animals. **D.,** A representative measurement of oxygen levels, using the oxygen telemetric device, in the kidney of a single rat for 3 consecutive days. The dark line represents the moving average of the raw data. **E.,** Phase graph shows the peak time of kidney oxygen levels. Data are presented as mean ± SD, for 5 individual animals monitored for several consecutive days. **F.,** Analysis of the oxygen differences between the nadir and zenith levels during the day. Data are presented as mean ± SD, for 5 individual animals monitored for several consecutive days. **G.,** and **H.,** Wild type mice were sacrificed at 4 h intervals throughout the day. Kidney (**G**) and Brain (**H**) were isolated and nuclear extracts were prepared and analyzed by SDS-PAGE and immunoblot with the indicated antibodies (each time point consists of a mix of 4 individual animals). Gray shading represents the dark phase, Zeitgeber Time (ZT), oxygen consumption (VO_2), * $p < 0.05$, *** $p < 0.001$. See also Figure S1.

Figure 2. Physiological oxygen cycles synchronize circadian clocks in HIF1α-dependent manner. A., A schematic illustration of the chambers with CO_2, O_2 and temperature control that were used for the experiments in Figures 2 and 3 (left panel). Representative measurements of O_2 and temperature using O_2/Thermo meter during an oxygen cycle of 12 h 5% and 12h 8% (right panel). CO_2 levels were maintained constant at 5% throughout the experiment. **B.,** A schematic depiction of the experimental protocol. The arrow indicates the

sampling starting point. **C.,** Hepa-1c1c7 cells were either exposed to oxygen cycles or maintained under constant oxygen levels and were harvested at 4 h intervals throughout the 'free running' period. Total RNA was prepared and mRNA expression levels of clock genes were determined by quantitative real-time PCR. **D.,** Following transfection with control or *Hif1α* siRNA, cells were exposed to oxygen cycles and were harvested at 4 h intervals throughout the 'free running' period. Total RNA was prepared and mRNA expression levels of clock genes were determined by quantitative real-time PCR. **E.,** Protein cell extracts were prepared under the same experimental setup as in D, and analyzed by SDS-PAGE and immunoblot with the indicated antibodies. **F.,** Following transfection with control or *Hif1α* siRNA, cells were placed under 8% O_2 for 2 days and then exposed to a short pulse of Dexamethasone (DEX). Cells were harvested at 4 h intervals 24 to 48 h from the Dexamethasone treatment. Total RNA was prepared and mRNA expression levels of clock genes were determined by quantitative real-time PCR. Real-time PCR data are presented as fold change relative to the lowest value with mean ± SD of 3 individual experiments. Circadian Time (CT). See also Figure S2 and Table S1.

Figure 3. Specific clock genes respond to changes in oxygen levels in HIF1α-dependent manner. A., A schematic depiction of the experimental protocol. The arrow indicates the sampling starting point. An oxygen cycle (cue) was applied after 3 days in constant 8% to minimize the potential effects of cell seeding and/or media change. **B.,** Hepa-1c1c7 cells were either exposed to oxygen cycle or maintained under constant oxygen levels and were harvested at 4 h intervals in the course of the oxygen cycle. **C.,** Following transfection with control or *Hif1α* siRNA, cells were exposed to oxygen cycle and were harvested at 4 h intervals in the course of the oxygen cycle. **D.,** Following transfection with control or *Cry2* siRNA, cells were exposed to oxygen cycles for 3 consecutive days and were harvested at 4 h intervals on day 4

throughout the 'free running' period (see schematic depiction of the experimental design in Figure 2B). **E.,** Following transfection with control or *Rorα* siRNA, cells were exposed to oxygen cycles for 3 consecutive days and were harvested at 4 h intervals on day 4 throughout the 'free running' period (see schematic depiction of the experimental design in Figure 2B). Total RNA was prepared and mRNA expression levels of clock genes were determined by quantitative real-time PCR and presented as fold change relative to the lowest value. Data are presented as mean ± SD of 3 individual experiments. Circadian Time (CT), Zeitgeber Time (ZT). See also Figure S3 and Table S1.

Figure 4. Low oxygen pulse accelerates the adaptation of mice in a jet lag protocol. Mice were housed under 12 h light-dark regimen for several days, subsequently the lighting schedule was 6 h advanced. **A.,** Representative double-plot actograms for the wheel-running activity of wild type mice housed either under constant 21% O_2 or exposed to 12 h of 16% O_2 prior to the shift in the lighting schedule. **B.,** The distribution of the number of days that took mice to adopt the new lighting schedule under the different oxygen regimens are presented together with the mean ± SD (7.4 ± 0.2 and 5.5 ± 0.3 days under constant 21% O_2 and 12 h of 16% O_2 respectively, p value 1.82E-06, n=28). **C.,** Representative double-plot actograms for the wheel-running activity of wild type mice housed either under constant 21% O_2 or exposed to 2 h of 14% O_2 following the shift in the lighting schedule. **D.,** The distribution of the number of days that took mice to adopt the new lighting schedule under the different oxygen regimens are presented together with the mean ± SD (7.3 ± 0.3 and 5.3 ± 0.2 days under constant 21% O_2 and 2 h of 14% O_2 respectively, p value 6.45E-06, n=16). **E.,** Representative double-plot actograms for the wheel-running activity of *Hif1α$^{+/+}$* and *Hif1α$^{+/-}$* littermates subjected to 6 h phase advance in the lighting schedule. **F.,** The distribution of the number of days that took the two different mouse strains to adopt the new lighting schedule are presented together with the

mean ± SD (6.5 ± 0.4 and 6.4 ± 0.4 days for $Hif1\alpha^{+/+}$ and $Hif1\alpha^{+/-}$ littermates respectively, p value 0.9, n=15). **G.,** Representative double-plot actograms for the wheel-running activity of $Hif1\alpha^{+/+}$ and $Hif1\alpha^{+/-}$ littermates exposed to 12 h of 16% O_2 prior to the shift in the lighting schedule. **H.,** The distribution of the number of days that took the two different mouse strains to adopt the new lighting schedule are presented together with the mean ± SD (5.1 ± 0.2 and 6.3 ± 0.3 days for $Hif1\alpha^{+/+}$ and $Hif1\alpha^{+/-}$ littermates respectively, p value 0.002, n=15). **I.,** Representative double-plot actograms for the wheel-running activity of $HIF1\alpha^{+/+}$ and $HIF1\alpha^{+/-}$ littermates exposed to 2 h of 14% O_2 following the shift in the lighting schedule. **J.,** The distribution of the number of days that took the two different mouse strains to adopt the new lighting schedule are presented together with the mean ± SD (4.9 ± 0.2 and 6.3 ± 0.2 days for $Hif1\alpha^{+/+}$ and $Hif1\alpha^{+/-}$ littermates respectively, p value 0.0004, n=15). **K.,** A schematic model for circadian clock resetting by oxygen through HIF1α. Clock components that respond to oxygen levels in HIF1α–dependent manner are colored in blue. The blue line marks the time mice were exposed to low oxygen levels. Time spans during which the lights were switched off are marked by gray shading. ** $p < 0.01$, *** $p < 0.001$, N.S - Non Significant. Zeitgeber Time (ZT). See also Figure S4.

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A.

| | Day 1 | Day 2 | Day 3 | Day 4 |
Constant
Cue

5% O₂
8% O₂

Cue

ZT= 0

B.

Clock *Bmal1* *Rev-erbα*
Rorα *Per1* *Per2*
Cry1 *Cry2* *Glut1*

Fold change

ZT

◆ Constant   ▲ Cue

D.

Free Running

Clock *Bmal1* *Rev-erbα*
Rorα *Per1* *Per2*
Cry1 *Cry2* *Dbp*

Fold change

CT

— O₂ rhythms siControl   -◆- O₂ rhythms si*Cry2*

C.

Clock *Bmal1* *Rev-erbα* *Hif1α*
Rorα *Per1* *Per2* *Hif2*
Cry1 *Cry2* *Glut1*

Fold change

ZT

— Cue siControl   ··▲·· Cue si*Hif1α*

E.

Free Running

Clock *Bmal1* *Rev-erbα*
Rorα *Per1* *Per2*
Cry1 *Cry2* *Dbp*

Fold change

CT

—◆— O₂ rhythms siControl   -■- O₂ rhythms si*Rorα*

A. WT   WT, 12hr 16% O$_2$ B.

C. WT   WT, 2hr 14% O$_2$ D.

E. *Hif1α* $^{+/+}$ *Hif1α* $^{+/-}$ F.

G. *Hif1α* $^{+/+}$, 12hr 16% O$_2$ *Hif1α* $^{+/-}$, 12hr 16% O$_2$ H.

I. *Hif1α* $^{+/+}$, 2hr 14% O$_2$ *Hif1α* $^{+/-}$, 2hr 14% O$_2$ J.

K.