Breath hold task induces temporal heterogeneity in electroencephalographic regional field power in healthy subjects

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Abstract

While the brainstem is in charge of the automatic control of ventilation, the cortex is involved in the voluntary control of breathing but also receives inputs from the brainstem, which influence the perception of breathing and the arousal state and sleep architecture in conditions of hypoxia/hypercapnia.

We evaluated in eleven healthy subjects the effects of breath hold (BH: 30 seconds of apneas and 30 seconds of normal breathing) and BH-related CO_2/O_2 changes on electroencephalogram (EEG) global field power (GFP) and regional field power (RFP) in 9 different areas (3 rostrocaudal sections -anterior, central, posterior- and 3 sagittal sections -left, middle, right) in the δ and α bands, by cross correlation analysis.

No significant differences were observed in GFP and RFP when comparing free breathing (FB) with the BH task. Within the BH task, the shift from apnea to normal ventilation was accompanied by an increase in the δ power and a decrease in the α power. The end-tidal pressure of CO_2 ($P_{ET}CO_2$) was positively correlated with the δ-band and negatively with the α- band with a positive time shift, while an opposite behaviour was found for the end-tidal pressure of O_2 ($P_{ET}O_2$). Notably, the time shift between $P_{ET}CO_2/P_{ET}O_2$ signals and cortical activity at RFP was heterogenous and seems to follow a hierarchical activation with the δ-band responding earlier than the α band.

Overall, these findings suggest that the effect of BH on the cortex may follow specific ascending pathways from the brainstem and be related to chemoreflex stimulation.

New & Noteworthy

We demonstrated that the end tidal CO2 oscillation causes oscillations of delta and alpha bands. The analysis of the regional field power evidenced that different cortical areas respond with different time delays to CO2 challenges. An opposite behaviour was found for the end-tidal O2. We can suppose that the different cortical time delay response likely express specific ascending pathways to the cortex generated by chemoreceptor nuclei in the brainstem.

Key words:

EEG, neural pathways, respiration, hypercapnia, hypoxia, chemoreflex

List of abbreviations:

BH: Breath hold, CA: Central apnea, CCF: cross correlation function, CO_2: Carbon dioxide, CV: coefficient of variation, EEG: Electroencephalography, FB: Free breathing, GFP: Global field power, H0: null hypothesis, LA: left anterior, LC: left central, LP: left posterior, MA: middle anterior, MC,: middle central, MP: middle posterior, O_2: Oxygen, OSA: obstructive sleep apnea, PCA: Principal component analysis, $P_{ET}CO_2$: End-tidal CO_2, $P_{ET}O_2$: End-tidal O_2, RA: right anterior, RC: right central, RFP: Regional field power, RP: right posterior, SpO_2: Oxygen saturation.

Introduction

Spontaneous breathing in mammals is a complex function under automatic control of the brainstem neural network (29). This network originates in the medulla, receives inputs both from the periphery and the cortex and is responsible for the background respiratory rhythm and the coupling of oxygen (O_2) consumption and carbon dioxide (CO_2) production to metabolic needs, a function known as chemosensitivity(19).

CO_2 is the primary chemical stimulus for alveolar ventilation and is mainly sensed by the central chemoreceptors (70-80% of CO_2 response in condition of normoxia). These receptors are mainly located in the medulla, and respond to pH and CO_2 variations, so that in condition of hypercapnia they cause an increase in ventilation resetting CO_2 and pH to steady state levels. On the other hand, peripheral chemoreceptors, located in the carotid bodies in humans, are mainly responsible of O_2 levels and the sensing of hypoxia, but also respond to CO_2 (20-30% of CO_2 response in condition of normoxia) and pH variations (12, 25).

Beyond their effects on ventilation and the autonomic outflow, the chemoreflex is known to have influences also on the cortex through specific ascending pathways (22, 39). Tracking this pathway is not only physiologically but also clinically relevant, since it is involved in the perception of breathing (i.e. dyspnea), alertness during wakefulness or arousability during sleep and it is implicated in conditions associated with oscillatory ventilation, such as obstructive sleep apnea (OSA) and central apneas (CA). Moreover, the possibility to pharmacologically or surgically modulate the chemoreflex has recently emerged in sleep disorder breathing, hypertension and heart failure (14, 24, 32, 36, 38, 40) and thus there might be the need to explore the effects of these novel interventions on cortical activity.

In this respect, the effects of CO_2/O_2 variations on brain activity has been mainly explored by using electroencephalography (EEG) (7, 34, 35, 49, 53, 54, 56). In humans, hypercapnia is known to cause an increase in the EEG global field power (GFP) in the δ-band (1–4 Hz), as well as a reduction in the α- band (8–13 Hz) (34, 35, 54, 56). This suggests that during hypercapnia, brain activity resembles a low arousal state (54, 56). Similar results were observed in condition of asphyxia, such as those induced by chocking, in which hypercapnia is accompanied by a various degree of hypoxia (41).

Variations in CO_2 and O_2 arterial levels may be experimentally induced by either administering different gas mixtures or by voluntary breath hold (BH). Despite having also some effects on cortical motor/sensory activity, BH has the advantage to be easy to perform, without requiring a

specific device respect to gas administration (30). BH initially requires conscious inhibition by the cortex of the brainstem network, but then allow a progressive increase in CO_2 and decrease in O_2 mimicking the respiratory dynamics of OSA and CA (23). In those conditions, a sinusoidal increase in CO_2 and decrease in O_2 is usually observed differently from the square wave increase to non-physiological O_2/CO_2 values generally obtained by gas administration. However, to stress the chemoreflex system in the right range of perturbation, the respiratory cycle time is fundamental, since the average apnea length of OSA/CA is approximately 30 seconds (15, 16, 37). Previous studies have used longer (80–225 seconds) or shorter (10 seconds) voluntary BH intervals (43, 45). The cross correlation between EEG GFP in δ- band and end-tidal CO_2 ($P_{ET}CO_2$) during 30 seconds of BH has been recently explored by our group, finding that the variation of $P_{ET}CO_2$ usually precedes the variations observed in the GFP (34). However, by looking at GFP, it is not possible to comprehend whether different cortical areas do respond to gas challenges in a different fashion.

This is of physiological interest, since if the cortex is responding to stimulation of the different group of chemoreceptors during gas challenges some heterogeneous temporo-spatial distribution of neural response is likely to occur. Indeed, different chemoreceptors usually operate around different response thresholds and with different time delays and have specific neuroanatomical connections with the cortex (11, 28, 31, 47). On the other hand, CO_2/O_2 changes may cause effects on cortical activity by either vasodilation or direct neural activation, independently from chemoreflex recruitment. If this alternative hypothesis is correct a rather uniform and homogeneous variation in cortical activity is to be expected. While it is impossible to unravel this question by looking at EEG GFP, the use of regional field power (RFP) analysis may instead shed light on this topic.

Therefore, in the current study, we aimed at studying by cross correlation analysis the cortical regional variations of EEG RFP in the δ and α bands related to hypercapnia and hypoxia induced by voluntary BH in healthy subjects.

Material and Methods

Eleven healthy subjects (all males, age 30±6 years) were recruited in the study. Six subjects derived from a previous study of our group in which only EEG GFP changes in the δ- band induced by BH were investigated and related to $P_{ET}CO_2$ (34), and thus this study it is partly a reanalysis of previous data. However, a larger population was enrolled in the current study focusing this time on the effects of BH on EEG RFP. Furthermore, the effects of $P_{ET}O_2$ and the changes observed in the α-band were incorporated in the analysis, differently from our previous work.

A 64-electrode EEG device was used (Compumedics Neuroscan, SynAmps RT) to record brain signals. Simultaneously with EEG acquisition, exhaled CO_2, O_2 and blood oxygen saturation (SpO_2) were recorded with a gas analyzer (Cosmoplus®; Novametrics) and a pulse oximeter (Pulsox-7; Minolta), respectively.

Two different tasks were performed. In the free breathing (FB) task subjects had to breathe normally for 6 minutes while lying down with eyes closed. In the BH task, the subjects had to breath normally for 1 minute and then alternate 30 seconds of BH performed after normal inspiration to 30 seconds of normal breathing for 5 cycles, for a total of 6-minute acquisition, still with eyes closed. Subjects were advised to start or stop the BH by touching their left leg. The same touching procedure was used during the FB task, to control for somatosensory potential confounders due to the instructions given to the subject.

The experimental protocol was approved by the Institutional Ethical Committee. The recordings were carried out in agreement with the Declaration of Helsinki. Written informed consent was obtained from all subjects.

EEG analysis

EEG signal analysis was already described in (34). Briefly, all channels were re-referenced to average signals and channels with low Signal-to-Noise ratio were excluded from the analysis. The impedance of all electrodes was checked and kept below 30 kΩ during all recordings to ensure a good signal quality. EEG signals underwent baseline correction, Hann pass band filter (1-30Hz), blink and cardiac artefacts detection and removal using a Principal Component Analysis (PCA) method (51). The global measure of EEG power expressed as GFP and the regional distribution of the EEG power in different brain areas was obtained as RFP for the δ (1-3 Hz) and α (8-13 Hz) bands. Specifically, for RFP, 9 areas were extracted dividing the scalp into 3 different sections (left L, middle M and right R) and further dividing into 3 rostrocaudal sections (anterior A, central C and posterior P) (35).

Physiological signal processing

The physiological signals were processed as described in (34). Briefly, SpO_2 was used to detect possible effects induced by the tasks on oxygen levels. The normal range of SpO_2 was considered to lie between 95% and 100%. The exhaled CO_2 and O_2 waveforms were used to estimate the $P_{ET}CO_2$ and $P_{ET}O_2$ time series respectively, as an estimate of arterial CO_2 and O_2 (33). Since no exhalation of CO_2/O_2 does occur during the voluntary apnea phase, the $P_{ET}CO_2$ and $P_{ET}O_2$ signals were

estimated by using a cubic spline model interpolating $P_{ET}CO_2$ and $P_{ET}O_2$ found before and after the cessation of breathing.

Cross Correlation Analysis

The cross-correlation function (CCF) was used to evaluate the similarity between $P_{ET}CO_2$/ $P_{ET}O_2$ and EEG power (both GFP and RFPs) as a function of different time lags. The CCFs were estimated using the method previously described (46). Briefly, the peak of CCF and the corresponding time lag were evaluated. In our analysis, if the maximum correlation occurred for negative time shift, the EEG power signal was considered to lead the $P_{ET}CO_2$/$P_{ET}O_2$ signal. On the other hand, if the maximum correlation occurred for positive time shift, the $P_{ET}CO_2$/ $P_{ET}O_2$ signal was considered to lead to the EEG signal. The CCF was estimated for time lags between -30 s and 30 s as proposed in (57). Furthermore, the weighted average CCFs were also estimated on the group of subjects under study.

Statistical analysis

Variations in $P_{ET}CO_2$/$P_{ET}O_2$ and RFP between the two task (FB, BH) were estimated by comparing their medians with a non-parametric Wilcoxon signed rank test (27), with the null hypothesis (H0) being that the differences between FB and BH tasks came from a distribution with zero median. A comparison between the phase of voluntary apnea and that of normal breathing within the BH task was also performed with a non-parametric Wilcoxon signed rank test (27). In order to assess at the group level how cross correlation between $P_{ET}CO_2$/$P_{ET}O_2$ and GFP or RFP varied between the two tasks (i.e. FB, BH), we compared their weighted-average cross correlation values with a non-parametric Wilcoxon signed rank test under the null hypothesis (H0) of no differences between the tasks. We controlled false-discovery-rate through the Benjamini-Yekuteli correction for multiple testing under dependency (4, 5). We assessed the statistical significance of cross-correlation analysis by means of a phase randomization approach (48). Specifically, for each subject, we generated n=1000 surrogates of $P_{ET}CO_2$/$P_{ET}O_2$, EEG δ power and α power (both global and regional) under the null hypothesis of no correlation between the time-series. Specifically, such surrogates preserved original time-series magnitude but with randomly distributed phase in range $(0,2\pi)$. Then, we evaluated the cross-correlation between surrogate time-series. Accordingly, at regional analysis we obtained for each scalp region *(s)*, for each frequency *(f)* and for each time-lag *(t)* a surrogate distribution of cross-correlation under the null-hypothesis of absence of correlation. Then, we associated to each observed cross-correlation *(s,f,t)* a p-value based on its position in the surrogate distribution. The same procedure was repeated for GFP analysis for each frequency and

for each time-lag. Finally, we controlled multiple testing *(α=0.05)* with the False-Discovery-Rate procedure described (4, 5). Accordingly, we obtained the critical *p-value* at which tests were considered significant.

Results

An oscillatory behaviour with larger variations in both $P_{ET}CO_2$ and $P_{ET}O_2$ was documented during BH as compared to FB (coefficient of variation, CV, for $P_{ET}CO_2$: BH 7.1% versus FB 2.2%, p=0.001; CV for $P_{ET}O_2$: BH 8.8% versus FB 2.6%, p= 0.008). No significant changes were instead observed in the SpO_2 signal between the 2 tasks (CV for SpO2, FB: 1.1% versus CV BH; 0.7%, p=0.11), and thus $P_{ET}O_2$ rather than SpO_2 was used for subsequent analyses.

The signal behaviour during FB and BH tasks in a sample subject for GFP in δ and α bands, $P_{ET}CO_2$ and $P_{ET}O_2$ is shown Figure 1S (https://doi.org/10.6084/m9.figshare.12040578), while the GFP average values in the whole group of subjects between FB and BH and, within the BH task, between the phases of apnea and normal breathing are reported in Table 1S (https://doi.org/10.6084/m9.figshare.12040578). The time courses of the CCFs estimated at group level for GFP during FB and BH tasks for $P_{ET}CO_2$ and $P_{ET}O_2$ are shown in Figure 2S and Table 2S and Figure 3S and Table 3S, respectively (see https://doi.org/10.6084/m9.figshare.12040578). As for $P_{ET}CO_2$, a positive correlation with the δ band and a negative correlation with the α band were observed for a positive time shift, while the opposite behaviour was instead observed for $P_{ET}O_2$.

Spectral maps and regional field power analysis

When comparing FB with the BH task considered as a whole, no significant differences were observed in the EEG spectral maps (Table 1 and Figure 1). Conversely, within the BH task, an increase in the δ power and a decrease in the α power was observed after the apnea phase (Table 1 and Figure 1). More precisely at the spectral maps, the same behaviour was observed in all regions but RA and RC in the δ-band, and LA, RA, RC and RP in the in the α- band (Table 1 and Figure 1). Each one of the 9 regions included in the EEG analysis had at least 2 channels, since the average number of electrodes removed from the analysis was 3 ± SD, and the spatial distribution of excluded electrodes was sparse.

The CCFs for the 9 cortical areas during FB and BH tasks for both the δ and α bands are shown in Figure 2 for $P_{ET}CO_2$ and in Figure 3 for $P_{ET}O_2$. During FB, the CCFs between $P_{ET}CO_2$ and RFP and $P_{ET}O_2$ and RFP were not significant. On the contrary, within the BH task, the CCF between

$P_{ET}CO_2$ and RFP was found to be positive in the δ band and negative in the α band for positive time shifts in all regions but RP for the δ band (not significant) and RA, RC and RP for the α band (positive correlations) (Table 2 and Figure 2b). The CCF between $P_{ET}O_2$ and RFP was found instead to be negative in the δ band and positive in the α band for positive time shifts in all cases but RA and RP for the δ band (positive correlations) (Table 3 and Figure 3b).

Notably, a different phase shift in the $P_{ET}CO_2$ concentration waveform and the EEG power waveform was observed in the different cortical regions (Video S1 see https://doi.org/10.6084/m9.figshare.12040578). In particular, in the δ band an earlier activation in the MA and MC (2 seconds of delay) was observed, with the LA, LC e MP showing the highest EEG delay (ranging from 7 to 9 seconds) . In the α band, an even more heterogeneous behaviour of cortical time responses to $P_{ET}CO_2$ variations among different regions was observed, with the right regions (RA, RC, RP) usually showing negative time delays and positive correlations, differently from the medial and left regions showing instead positive time delays and negative correlations. In the latter regions, a longer time shift and a lower time dispersion was documented in the α band as compared to the δ band (range 14-16 seconds vs 2-9 seconds) (Figure 2b, Table 2). A similar trend with an opposite sign was observed for CCFs between $P_{ET}O2$ and RFP (Video S2 see https://doi.org/10.6084/m9.figshare.12040578) (Figure 3b, Table 3).

Discussion

In this study, the cortical responses to BH were evaluated in healthy subjects, both globally (average across all electrodes) and, for the first time, in nine different regions (3 rostrocaudal sections -anterior, central, and posterior- and 3 sagittal sections -left, middle, and right) for 2 frequency bands of interest (i.e. δ and α bands). No significant changes were observed in the cortical activity by comparing FB to BH, but, within the BH manoeuvre, a significant variation was observed by comparing the apnea phase with the normal ventilation phase. Notably, $P_{ET}CO_2$ was positively correlated with the δ-band and negatively with the α- band (apart from the right regions) for positive time shifts, while $P_{ET}O_2$ was negatively correlated with the δ-band and positively correlated with the α- band. This is logical considering the counter phase oscillation of $P_{ET}CO_2$ and $P_{ET}O_2$ during the two phases of BH. Most importantly, a different time shift between $P_{ET}CO_2$ and $P_{ET}O_2$ envelopes and the EEG RFP signal in the 9 cortical regions was observed, suggesting that the effect of BH on the cortex may follow specific ascending pathway from the brainstem due to chemoreflex stimulation.

Although coherent and significant trends were observed across all subjects, we reported only the results of the analysis at group level, which are more consistent, considering the simulated test results (34) and the observed percentage of missing data segments in each subject. Our findings are in line with previous studies, in which an increase in the δ band and a reduction in the α band was found throughout the whole brain in hypercapnic condition (54, 56). Similar findings were also observed in patients with spontaneous apneas, such as patients with OSA and CA (17, 50) Interestingly, in the study by Wang and colleagues (54), three different conditions were compared: 1) hyperoxic-hypercapnia; 2) hypoxic-hypercapnia; 3) normocapnic-hypoxia. Both hyperoxic-hypercapnia and hypoxic-hypercapnia led to an increase in the δ-band power and to a decrease in the α- band, without any significant difference between the two trials. Most notably, hypoxic-normocapnia had no effect on the two bands. These findings suggest that CO_2 rather than O_2 seems the main driver of EEG changes also during BH.

Considering that BH is a complex task, which involves, beyond task-related oscillations in the respiratory gases, also a change in the motor and sensory cortical activity, we cannot exclude that the observed changes may be due to the task commitment rather than the observed gas variations. However, considering that similar results were obtained by gas administration (54, 56) and observed in spontaneous apneas (17, 50), this potential interpretation seems less likely, although it cannot be completely excluded especially when RFP analysis is taken into consideration.

In some specific areas associated with task engagement, that are usually in the right hemisphere (2, 55) and in the α band, which is commonly associated with higher arousal state (56), the results may be partially influenced by the task. However, the sinusoidal shape perfectly following the CO_2 /O_2 behaviour observed at the cross-correlation analysis even at RFP analysis seems again to conflict with this possible interpretation, at least with spatio-temporal resolution of the study. A way to explore this topic would be to ask the subject to think about performing a BH without actually doing it (no gas changes) or to perform the phase of normal breathing imposing a paced rhythm (both apnea and normal ventilation under voluntary control). Furthermore, focusing on specific areas known to be associated with task commitment and using high resolution EEG may be of additive value in this respect.

The main novelty of the current study is surely represented by the analysis of RFP, that has never been performed so far neither in BH studies, nor in experiments involving gas administration. This kind of analysis may help us to understand another relevant issue. Indeed, it is still not completely clear whether the cortical activation, which follows a gas challenge would be mediated by the chemoreflex or related by a direct effect of hypercapnia or hypoxia on cortical neurons. Potentially

a change in the EEG activity may also derive from the vasodilatory effect due to hypercapnia and hypoxia. If the latter two hypotheses were correct, a rather uniform and homogeneous change in cortical activity would have emerged. On the contrary, both looking at the spectral maps and the RFP-related cross correlations a heterogeneous temporo-spatial distribution was found.

Another novelty of this study stands in the analysis of the temporal distribution of RFP responses to to BH. Specifically, following an increase in $P_{ET}CO_2$ the first cortical areas which showed an increased activity were the middle central and middle anterior, with the wave of cortical activation then irradiating to other more lateral and posterior areas (see also Video S1 https://doi.org/10.6084/m9.figshare.12040578). The temporal trends observed in the δ band are biologically plausible considering the neuroanatomy of the ascending pathways originating from the brainstem neural network (1, 9, 11, 28). The time delay must be interpreted as a phase shift between the CO_2 waveform and the EEG waveform. While for the δ band the time shift ranges from 2 to 9 seconds, the time shift is usually longer and narrower (14-16 seconds) for the α- band and might thus reflect a secondary variation from a hierarchical standpoint.

Finally, the correlation between $P_{ET}CO_2/P_{ET}O_2$ and cortical activity is almost completely lost during FB across the different regions of interest. At most, the sign of the correlation (generally weak) is reverted in some cortical regions as compared to BH. Indeed during wakefulness and in FB conditions, the pre-Bötzinger complex in brainstem acts as the pacemaker of respiration controlling respiratory motoneurons, respiratory muscles and finally pulmonary ventilation, and it is poorly influenced by the chemoreflex (21). Therefore, any cortical activity related to e.g. speaking, eating, or emotions (descending pathways) may cause variations in ventilation and, thus, in CO_2 and O_2 operating on the plant (lung) gain (26).

Study limitations

EEG channel impedance was kept below 30 kΩ on average across subjects. This value is relatively high compared to the typical values, usually set below 10 kΩ. However, with modern high input-impedance amplifiers and accurate digital filters for power line noise, it has been demonstrated that high-quality EEG can be successfully recorded with impedance values up to 40 kΩ (20). Thus, considering the high input impedance (> 10 GΩ) of our EEG system, and the homogeneity of the EEG signal across the different electrodes of the EEG cap for each subject, we are confident that the signal was accurately captured at the scalp surface. Furthermore, considering the group under study, all subjects had similar values of impedance, and we observed coherent cross correlation courses. Finally, the quality of EEG signals was confirmed by highly experienced neurologists (MM, EB).

The circulation delay related to the increase in venous CO_2 (metabolism), the transit through the lung, the delivery to the central nervous system and the diffusion into the cerebrospinal fluid was not taken into account in this study. Indeed, this information is difficult to obtain in humans in vivo. Our group is currently developing novel methods to assess the circulatory time delay (unpublished data) in humans. These methods could be used in the future to correct for differences in cardiac output, especially when moving to patients in whom a greater variability in cardiac hemodynamic and circulatory delay is likely to occur. Moreover, the analysis of subcortical areas by deep source analysis or functional magnetic resonance imaging may help to understand some of these relevant questions.

Finally, in our study we limited our observation to the effects of voluntary BH on GFP and RFP. We have not considered the motor component of the task and other possible effects such as respiratory sensation that includes air hunger, chest tightness, effort of breathing, an urge-to-cough, urge-to-sneeze, sense of suffocation (13). Indeed, respiratory sensation involves neural pathways in the pons, midbrain, hypothalamus, amygdala, cingulate, parahippocampal and fusiform gyrus anterior insula, pre-supplementary motor area, middle frontal gyrus (6, 8, 13). In previous EEG studies dyspnea was evaluated through variations of respiratory evoked potential elicited by inspiratory occlusion and inspiratory resistive load (42, 52). However, dyspnea was not a topic of interest in the current physiological study, and the duration of BH was short enough not to create discomfort in healthy subjects. Furthermore, subjects have been preliminary trained to correctly perform the task, and the acquisitions did not begin until they were comfortable to perform BH. None of the subjects actually reported dyspnea or discomfort when executing the BH task and therefore we believe that the mental distress was rather low. Future studies could focus on this topic, by either increasing BH duration or selecting population in which the symptom is more likely to occur during a 30 second BH, such as patients with heart failure or chronic obstructive pulmonary disease.

Conclusion

In healthy subjects, a different behaviour of $P_{ET}CO_2$, $P_{ET}O_2$ and EEG power is observed during FB and BH task. During FB and in awake conditions, the cortex is sending signals to the brainstem, changing ventilation and causing subtle CO_2/O_2 variations. During BH task, the greater oscillatory changes in CO_2 and O_2 seems to cause consequent variation in cortical activity, with antiphase oscillation in the δ band (positive correlation with CO_2, negative correlation with O_2) and in the α

band (negative correlation with CO_2, positive correlation with O_2). At regional analysis, a specific temporal pathway of cortical activation may be identified in the δ band, with the earliest activation being observed centrally and a subsequent cortical propagation toward lateral and posterior regions with some delay. This seems also to apply to the α band, which is suppressed in an heterogenous fashion but with longer delays as compared to the δ band. This characteristic behaviour of cortical response to CO_2/O_2 variations could suggest a specific chemoreflex-mediated stimulation of the cortex through specific ascending pathways (especially in the δ band) and also the presence of cortico-cortical interactions (δ band changes preceding α band changes). The latter point should be more deeply investigated in future studies, by using multivariate measures of brain connectivity, as partial directed coherence or directed transfer function, applied to brain sources (3, 10, 18). Furthermore, the investigation of subcortical sources integrating different methodologies such as functional magnetic resonance imaging, could reveal the involvement of deep structures along with the corresponding pathways (44). These methodological advancement may be also exploited when designing future studies in which a specific group of chemoreceptor may be stimulated (i.e. reverse microdialisys or optogenetic stimulation in animals) or suppressed by specific drugs (also in humans) (24).

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Figure Legends

Figure 1: EEG spectral maps EEG maps in δ and α bands. Four different conditions are show: 1) Free breathing task, 2) Breath hold task, 3) Apnea phase within Breath hold task, 4) Respiratory phase in Breath hold task .

Figure 2 : Time courses of the cross-correlation functions as a function of the time shift in FB task and BH task between nine different brain areas (LA= left anterior, MA= middle anterior, RA= right anterior, LC= left central, MC= middle center, RC= right central, LP= left posterior, MP= middle posterior, RP= right posterior) and PETCO2. δ band results are reported in red dotted lines and α band results are in blue dashed lines. P-values are shown for maximum and minimum value of correlation. In bold, the strongest significant correlation coefficient. The critical values for significance after Benjamini-Yekuteli correction is p_crit = 0 for FB and p_crit=0.041 for BH (4,5).

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