# Depression of Cortical Activity in Humans by Mild Hypercapnia

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**Abstract:** The effects of neural activity on cerebral hemodynamics underlie human brain imaging with functional magnetic resonance imaging and positron emission tomography. However, the threshold and characteristics of the converse effects, wherein the cerebral hemodynamic and metabolic milieu influence neural activity, remain unclear. We tested whether mild hypercapnia (5% $CO_2$) decreases the magnetoencephalogram response to auditory pattern recognition and visual semantic tasks. Hypercapnia induced statistically significant decreases in event-related fields without affecting behavioral performance. Decreases were observed in early sensory components in both auditory and visual modalities as well as later cognitive components related to memory and language. Effects were distributed across cortical regions. Decreases were comparable in evoked versus spontaneous spectral power. Hypercapnia is commonly used with hemodynamic models to calibrate the blood oxygenation level-dependent response. Modifying model assumptions to incorporate the current findings produce a modest but measurable decrease in the estimated cerebral metabolic rate for oxygen change with activation. Because under normal conditions, low cerebral pH would arise when bloodflow is unable to keep pace with neuronal activity, the cortical depression observed here may reflect a homeostatic mechanism by which neuronal activity is adjusted to a level that can be sustained by available bloodflow. Animal studies suggest that these effects may be mediated by pH-modulating presynaptic adenosine receptors. Although the data is not clear, comparable changes in cortical pH to those induced here may occur during sleep apnea, sleep, and exercise. If so, these results suggest that such activities may in turn have generalized depressive effects on cortical activity. *Hum Brain Mapp 33:715–726, 2012.* © 2011 Wiley Periodicals, Inc.

**Key words:** cerebral blood flow; cerebral metabolic rate for oxygen; blood oxygenation level-dependent response; functional magnetic resonance imaging; magnetoencephalography; temporal lobe; prefrontal cortex; occipital lobe; carbon dioxide; arterial spin labeling

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## INTRODUCTION

The use of functional magnetic resonance imaging to localize brain activation during cognition rests on the relationship between neural activity and the blood oxygenation level-dependent (BOLD) response. According to the standard model [Buxton et al., 2004], neural activity uses energy and thus increases oxidative metabolism and the cerebral metabolic rate for oxygen ($CMRO_2$). The increase in $CMRO_2$ is accompanied by an increase in cerebral blood flow (CBF) and cerebral

blood volume that is at least twice as large [Davis et al., 1998; Fox and Raichle, 1986; Hoge et al., 1999]. Through this decoupling, the resulting net decrease in deoxyhemoglobin concentration produces the increased BOLD signal.

The characteristics of the converse effect, of changes in cerebral hemodynamics and other aspects of the metabolic and ionic milieu on neuronal activity, are less clear [Moore and Cao, 2008]. One method to explore this issue is to induce hypercapnia by breathing air enriched in $CO_2$. Carbon dioxide acts as a vasodilator and increases CBF by about 50% at 5% $CO_2$ concentrations [Kim et al., 1999], probably via its effects in decreasing intracerebral pH although the mechanisms are not fully understood [Ainslie and Duffin, 2009]. Clinically, it has long been known that decreasing arterial $CO_2$ levels through hyperventilation can provoke seizures in epileptic patients [Foerster, 1924], whereas hypercapnia can suppress them [Gibbs and Gibbs, 1950]. A more direct measure of the effects of hypercapnia on brain electrical activity is provided by the electroencephalogram (EEG), which is generated by transmembrane currents due to synaptic activity and related active membrane channels [Murakami et al., 2003; Niedermeyer and Lopes da Silva, 1999]. Aram and Lodge [1987] showed that in-slice preparations of rat cingulate cortex, which increased the levels of $CO_2$, caused a pH-mediated reduction in spontaneous EEG. In awake humans, the total power of spontaneous EEG across frequencies from 1 to 64 Hz has been reported to be reduced at moderate levels of hypercapnia [Bloch-Salisbury et al., 2000]. Similarly, in humans under general anesthesia, Kalkman et al. [1991] found significant increases in the alpha (8–12 Hz) and beta (13–30 Hz) bands of the spontaneous EEG after hyercapnic challenge (PaCO$_2$ $\sim$ 50 mm Hg), but not in the delta (1–4 Hz) or theta (5–7 Hz) frequency ranges. However, under apparently similar levels of hypercapnia, Halpern et al. [2003] reported alpha band power increases and delta (1–4 Hz) decreases, whereas in animals under chronic hypercapnia, Matakas et al. [1978] reported significant alpha decreases.

Most EEG power is in the lower frequency bands, which often are dominated by alpha and other rhythms that are inversely correlated with alertness, arousal, and BOLD signal [Feige et al., 2005; Goldman et al., 2002]. Thus, they are problematic as indicators of activation. Recently, Zappe et al. [2008], working in anesthetized monkeys, found that moderate levels of hypercapnia induced by 6% $CO_2$ resulted in decreased spontaneous beta and gamma activity and multiunit activity, whereas activity in the theta and alpha remained unchanged. Simultaneous BOLD and blood volume measurements showed an increase during hypercapnia. Administration of 3% $CO_2$ produced only a trend toward reduced spontaneous activity. They note that 3% $CO_2$ in the artificially ventilated anesthetized monkey may produce cerebrovascular effects more comparable to 5% $CO_2$ in the freely breathing unanesthetized human.

Event-related potentials (ERPs), time-locked averages of EEG with respect to a stimulus, are typically well correlated with the local BOLD response [Arthurs et al., 2006]

and thus may provide a less ambiguous measure of the neural response to hypercapnia. ERPs are volume-conducted local field potentials, which are closely correlated with the BOLD response in many but not all experimental situations [Devor et al., 2003; Logothetis and Wandell, 2004]. In apparently the only study thus far published of ERPs in humans during moderate hypercapnia, no significant change was found [Bloch-Salisbury et al., 2000]. In contrast, intracranial recordings from human patients during presurgical evaluation for epilepsy found substantial effects of mild hypercapnia on the firing rate of medial temporal lobe neurons [Halgren et al., 1977]. These results may have been influenced by pathology or medication effects. However, they are consistent with recent recordings of depressed monosynaptic responses in rat hippocampal slices under mild hypercapnia [Dulla et al., 2005]. Similarly, Jones et al. [2005] examined the response of the rat barrel cortex to electrical stimulation of the whisker pad under urethane anesthesia. Compared to an average of the pre- and posthypercapnic periods, 5% $CO_2$ evoked a nonsignificant 6% decrease in both MUA and CSD, whereas 10% $CO_2$ evoked a 35% decrease in CSD and a 35% decrease in MUA, both highly significant. Because the hypercapnic challenge for BOLD calibration is usually 5%, these data could be taken to support the assumptions of that technique. However, it is unclear whether anesthesia, species, or cortical location might result in a different threshold for hypercapnic effects on neuronal processing.

In addition to its critical relevance for modeling the neural basis of the BOLD response [Chiarelli et al., 2007b], the effects of mild hypercapnia on neural activity are also important in understanding the effects of many medical conditions such as sleep apnea and emphysema, where paCO$_2$ may be considerably removed from the norm (paCO$_2$ $\sim$ 40 mm Hg) [Kaila and Ransom, 1998]. Even such mundane activities as sleeping, exercise, and travel to high altitudes may alter paCO$_2$ [Ainslie and Duffin, 2009; Phillipson and Bowes, 1986; Richerson, 2004]. Indeed, the potential neural effects of very mild hypercapnia suggest that during cognitive and sensory processing, neural activity may not only influence hemodynamics but also those hemodynamic effects may feed back to influence neural activity [Moore and Cao, 2008].

In the current study, we recorded the magnetoencephalogram (MEG) to test the hypothesis that hypercapnia, as induced by breathing 5% $CO_2$, affects neural activity in conscious task-performing humans. MEG is similar to EEG in being directly related to transmembrane currents, but has higher spatial resolution and has complementary sensitivity to cortical generators [Cohen and Cuffin, 1983; Murakami et al., 2003]. Specifically, we tested if early sensory evoked activity in visual and auditory cortices and late cognitive activity in fronto-temporal association cortices during language processing were decreased by mild hypercapnia. We also tested if mild hypercapnia decreases evoked or spontaneous spectral power in different frequency bands. Across measures, locations, and latencies,
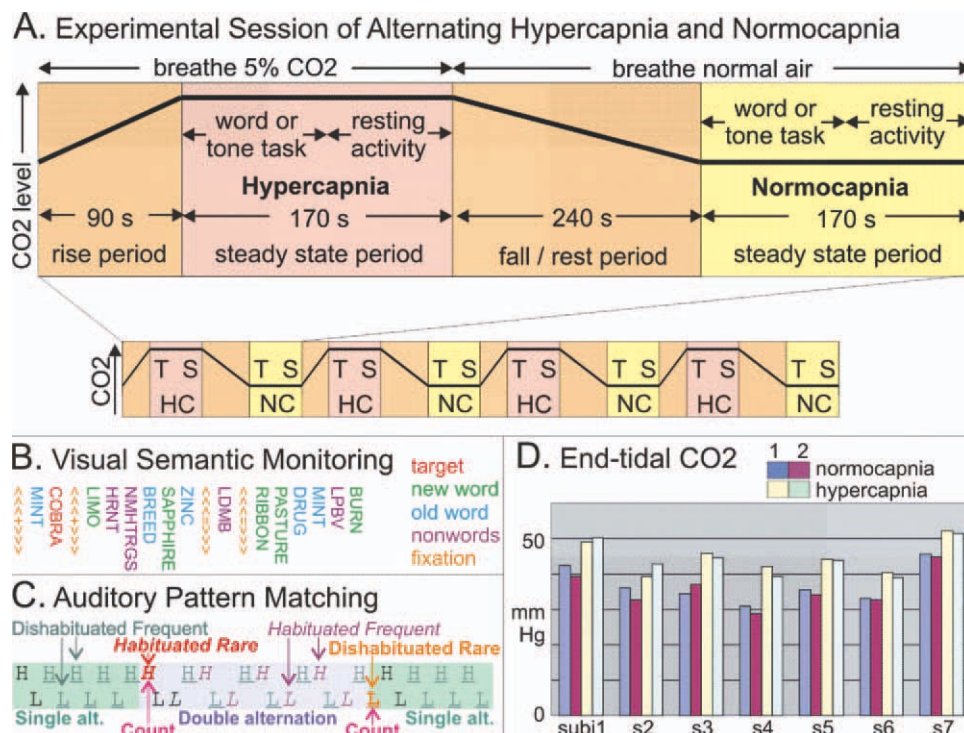
**Figure 1.**

Methods. **A:** Experimental session. A typical 48-min session comprising four hypercapnic (HC) and four normocapnic (NC) periods, with task-evoked (T) and spontaneous (S) MEG collected in each period. **B:** Example sequence from the Visual Semantic Monitoring Task. The subject detected animal names (targets) randomly interspersed among rapidly presented (SOA = 400 ms) words (either presented once, new, or presented many times after pre-exposure, old), nonpronounceable nonwords (npnw, consonant strings), or fixation ($\ll+\gg$). **C:** Example sequence from Auditory Pattern Monitoring Task. The subject counts sequence changes between single and double alternation of high (H, 500 Hz) and low (L, 250 Hz) 50 ms tones, SOA = 500 ms. **D:** Endtidal $CO_2$ levels obtained in the seven subjects during two cycles of normo- and hypercapnic conditions. Endtidal $CO_2$ concentrations increased by an average of ~8 mm Hg during HC. Task performance was >90% in all conditions and did not differ between HC and NC.

mild hypercapnia was found to have a consistent depressive effect on neural activity. We interpret these findings as reflecting a homeostatic mechanism and evidence of modulation of neural activity by hemodynamics.

## METHODS

### Participants and Recording procedures

Seven healthy right-handed volunteers free of neurological conditions (4 females, mean age: 25.4 years, standard deviation: 3.8 years) served as subjects. MEG signals were recorded from 306 channels at 0.1–1000 Hz using a Neuromag-Vectorview Instrument (Elekta, Stockholm) with a magnetometer and orthogonal pairs of planar gradiometers at each of 102 locations over the entire scalp.

Subjects were fitted with a breathing mask (H. Rudolph, Missouri, USA), which covered the entire nose and was fitted with a mechanism that prevented rebreathing of exhaled air. A pneumatic switching valve alternates breathing air between normal room air and humidified medical grade air with 21% $O_2$, 5% $CO_2$, and 74% $N_2$ through a low-resistance valve connected to a Douglas bag. Respiration and end-tidal $CO_2$ (ETCO_2) were recorded throughout the experiment (CardioPulmonary Technologies, Sussex, WI). Hypercapnic-breathing blocks consisted of subjects breathing a 5% $CO_2$ air mixture during a 90-s rise time and a steady-state $CO_2$ level for 120 s (see Fig. 1A). Under these conditions, the ETCO_2 plateau saturation in humans is reached after ~30 s of hypercapnia [Cohen et al., 2002].

During these periods, subjects were tested on the sensory and cognitive tasks, after which a 240-s rest period of normocapnic breathing followed to attain baseline $CO_2$ levels. Alternating normocapnic-breathing blocks followed the same pattern with the exception that subjects breathed ambient room air. To avoid order effects, three subjects were studied under normocapnic conditions first, and four subjects were tested under hypercapnic conditions first.

After each experiment, subjects were debriefed and asked if they were able to tell normocapnic from hypercapnic conditions. No subject reported a noticeable difference. This design allowed us to measure the effects of increased $CO_2$ levels in the blood on neuronal population activity across a wide range of sensory and cognitive tasks.

### Stimuli and Task

During repeated, counter-balanced HC/NC cycles with steady-state ETCO$_2$ levels, subjects were tested on visual semantic monitoring ($N = 7$) and auditory pattern monitoring ($N = 5$) while recording 306 channels of MEG. As is shown in Figure 1A, a given session consisted of four presentations of a single task during HC/NC/HC/NC or NC/HC/NC/HC periods. Fifteen seconds of spontaneous activity during rest with eyes open and fixated on a target were collected at the end of each task period.

### Visual Semantic Monitoring

During the visual task, subjects were presented with a continuous string of visual novel words, repeated words, nonpronounceable consonant strings, and fixation crosses at 400-ms stimulus onset asynchrony (see Fig. 1B). Four hundred words from each category were presented to ensure a high-signal-to-noise ratio. The sequence of stimulus conditions was balanced, so that each condition was preceded by every other condition with equal likelihood. In addition, 80 target words referring to animals were presented, and subjects were instructed to make a semantic decision by pressing a response button. Previous studies have shown that similar tasks evoke an N400 component that is associated with lexicosemantic word encoding [Kutas and Van Petten, 1994]. The N400m is maximal in gradiometers over the left anterior temporal and postero-ventral prefrontal cortices [Dale et al., 2000] where it is generated [Halgren et al., 1994a,b]. The N440m is larger to novel words, which require more sustained processing [Dale et al., 2000]. Visual words also evoke an M100 over occipital areas related to sensory processing.

### Auditory Pattern Discrimination

Subjects engaged in an auditory task (Fig. 1C) where they counted shifts in the ongoing pattern of tone sequences. All tones were either high (H; 500 Hz) or low (L; 250 Hz) pitched. Sequences were either single-alternation (i.e., H-L-H-L-...) and double-alternation (i.e., H-H-L-L-...). The tones had a duration of 50 ms and were presented at an SOA of 500 ms. A total of 528 tones were presented during each capnic condition. Unlike the typical "auditory oddball task," this design allows the effects of rare sequence shifts to be observed unconfounded by stimulus characteristics or dishabituation [Buxton, 2002]. Previous work demonstrates that rare pattern shifts evoke a sequence of fields from 200 to 400 ms mainly generated in temporal and frontal association areas and corresponding to the cognitive N2, P3a, and P3b components that have been extensively studied in scalp and intracranial ERPs [Halgren et al., http://www.ncbi.nlm.nih.gov/pubmed/20665718].

### Spectral Analysis

Artifact rejection of non-neuronal sources was performed on all single trials independent of experimental or capnic condition. Spectral analysis was performed on individual trials and spontaneous data (sampling frequency: 250 Hz, highpass filter: 0.1, and lowpass filter: 100 Hz) using the FieldTrip toolbox (F.C. Donders Centre: http:www.ru.nl/fcdonders/fieldtrip). Time-frequency responses were estimated with Morlet wavelets (width = 5) from 1 to 100 Hz at a 1-Hz frequency resolution and divided into theta (5–7 Hz), alpha (8–12 Hz), beta (13–24 Hz), low gamma (25–55 Hz), and high-gamma (65–95 Hz) frequency bands. All spectral analyses were performed on single trials and single subjects before creating averages and grand-average.

### RESULTS

We examined the effects of hypercapnia on evoked and spontaneous MEG activity.

### Endtidal CO$_2$

Mean endtidal $CO_2$ values were calculated for each individual from the presentation of the first stimulus to the last stimulus during HC and NC conditions (see Fig 1D). The average NC–HC difference (8 mmHG) is similar to other reports involving a 5% $CO_2$ challenge in humans [Howden et al., 2004].

### Behavioral

There was no significant difference between hypercapnia and normocapnia for either accuracy or reaction time in the visual semantic-monitoring task and no significant difference for accuracy in the auditory pattern matching task. Reaction time in the visual semantic-monitoring task under normocapnia was $655 \pm 22$ ms (mean $\pm$ standard deviation) and under hypercapnia was $690 \pm 49$ ms, a difference that was not significant (two-tailed, paired $t$-test; $p = 0.137$). Response accuracy on this task was $82\% \pm 13\%$ under normocapnia and $89\% \pm 5\%$ under hypercapnia, again a difference that was not significant (two-tailed, paired $t$-test; $p = 0.125$). Accuracy on the auditory pattern-matching task was $96\% \pm 2\%$ under normocapnia and $94\% \pm 1.5\%$ under hypercapnia, a difference that was not significant (two-tailed, paired $t$-test; $p = 0.173$). Breathing rate per minute during normocapnia was $17.2 \pm 1.9$ and during hypercapnia $17.7 \pm 1.3$ (mean $\pm$ standard
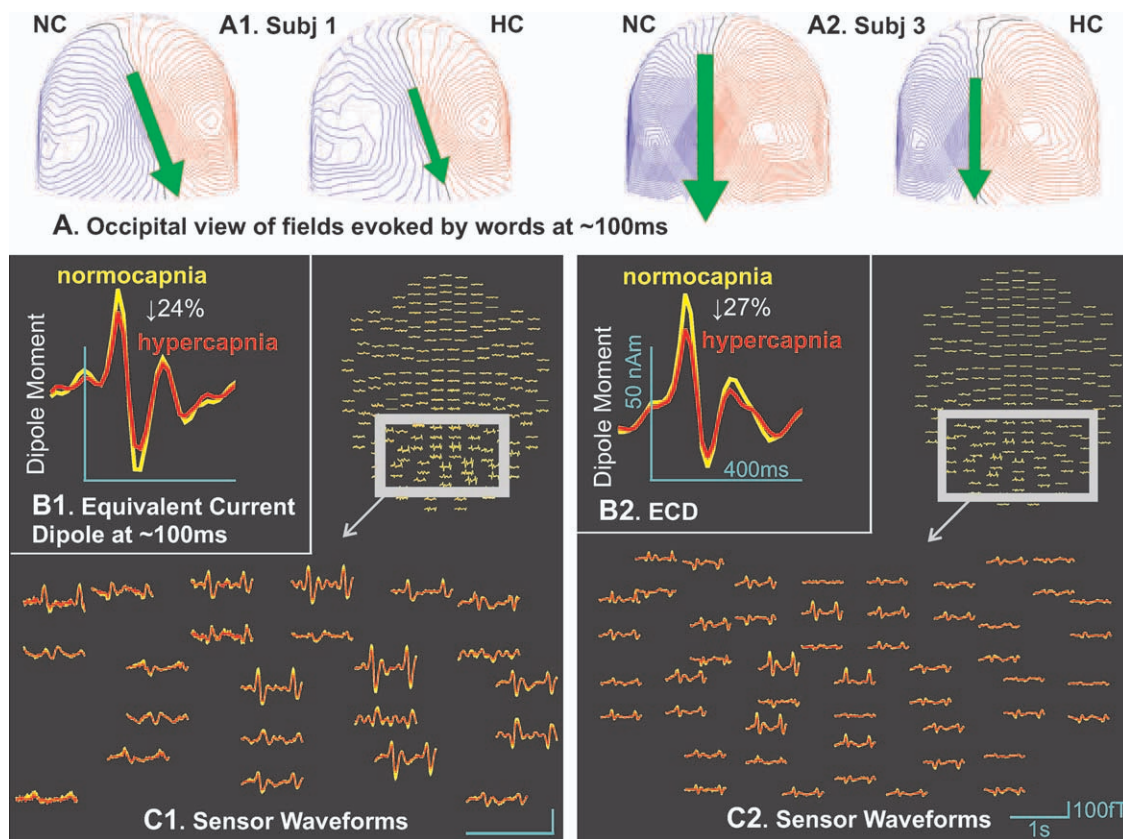
**Figure 2.**

Effects of hypercapnia on early occipital MEG fields evoked by words. **A:** Occipital view of MEG fields evoked by visual words during semantic monitoring at ~100 ms after word onset. Iso-field lines for inward flux are blue; outward flux are red visual (**B**). Equivalent current dipole fits to the occipital field at its maximal amplitude in the Visual Semantic Monitoring task, ~100 ms after stimulus onset. Hypercapnia significantly decreased dipole strength by ~20%. **C:** Average waveforms at the 204 MEG gradiometers to visual words during normocapina (yellow) and hypercapnia (red). All trials except fixation are included in these averages. Data from two subjects (numbers 1 and 3) are displayed.

deviation), a difference that was not significant (two-tailed, paired $t$-test; $p = 0.278$).

## Hypercapnia Attenuates MEG Fields Evoked by Visual Words

Analyses were performed on both sensory (M100) and cognitive (N400m) components of the averaged event-related field (ERF). A grand average of magnetoencephalo-gram (MEG) activity to novel words is shown in Figure 3A. Visually presented words evoked a strong dipolar pattern peaking at about 100 ms over the occipital lobe in all subjects (Fig. 2A). This pattern, the M100 evoked field, is typically observed in visual experiments with MEG. To quantify the effects of capnic state on neural response in different brain areas, an equivalent current dipole was fitted to the occipital field of the M100 at its maximal amplitude on the combined data from the normocapnic and hypercapnic conditions in each of the seven subjects. Examples from two subjects are shown in Figure 2B. Goodness of fit for the M100 response was 88% (std.dev. 4%). Dipole strength was then estimated separately in the normocapnic and hypercapnic conditions. Hypercapnia significantly decreased dipole strength by 20.3% (s.d. 5.5) (Table I) ($p < 0.005$, paired $t$-test, two-tailed).

Late peaks in the MEG fields appear larger under nor-mocapia than hypercapnia. Data were combined across channels and subjects in order to quantify this effect. Because the fields can be either positive or negative, the root mean square (RMS) was calculated across gradiome-ter sensors before averaging. Figure 3B shows the time course of the RMS averaged across the left frontotemporal sensors and all subjects. Our results show that the RMS

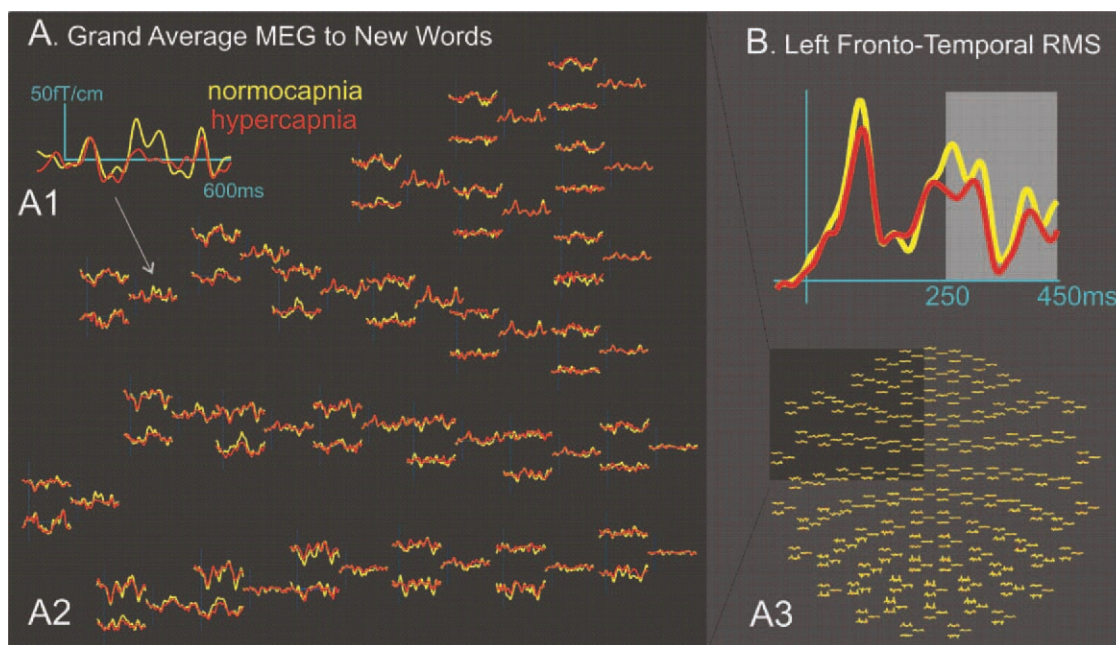**TABLE I. Effects of hypercapnia on MEG fields evoked by visual words**

| Subject | Normocapnic | | Hypercapnic | | HC-induced decrease in dipole moment (NC–HC)/NC (%) |
|---|---|---|---|---|---|
| | Dipole moment (nA m) | Goodness of fit (%) | Dipole moment (nA m) | Goodness of fit (%) | |
| 1 | 37 | 82 | 28 | 82 | 24 |
| 2 | 30 | 85 | 26 | 84 | 14 |
| 3 | 45 | 96 | 33 | 91 | 27 |
| 4 | 36 | 92 | 27 | 87 | 26 |
| 5 | 27 | 93 | 22 | 87 | 18 |
| 6 | 13 | 89 | 10 | 84 | 21 |
| 7 | 20 | 88 | 18 | 85 | 13 |

calculated at 250 and 450 ms after word onset is consistently larger during normocapnia compared to hypercapnia [$t(6) = 2.1$, $p < 0.001$].

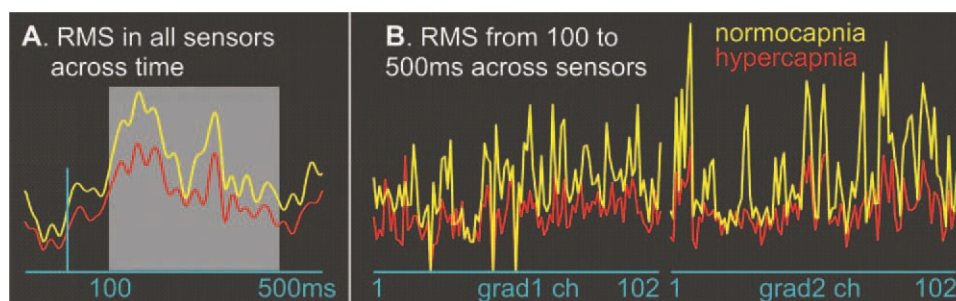## Hypercapnia Attenuates MEG Fields Evoked by Tone Pattern Shifts

Five of the subjects performed a pattern detection task where rapidly presented tones occasionally shifted between single and double alternation (Fig. 1C). RMS of differential magnetoencephalogram (MEG) activity evoked by rare tone pattern shifts when compared with control stimuli was calculated from individual trials for each subject across all 204 gradiometer sensors at each latency and then averaged across subjects. As is shown in Figure 4A, the RMS activity during the normocapnic periods was compared to the hypercapnic periods at all latencies. RMS activity was also averaged in each gradiometer channel between 100 and 500 ms after tone onset. As is shown in Figure 4B, RMS showed a significantly higher amplitude during the normocapnic when compared with the hypercapnic periods ($n = 5$, paired $t$-test, two-tailed, $p < 0.01$).



**Figure 3.**
Effects of hypercapnia on late frontotemporal MEG fields evoked by words. **A:** Grand average across seven subjects of response to all novel words. An example left frontotemporal sensor (**A1**) is shown, drawn from the entire set of left frontotempoal sensors (**A2**), which are in turn drawn from the entire sensor array (**A3**). **B:** Root mean square activity averaged across all left frontotemporal sensors. Late activity between 250 and 450 ms after word onset was chosen for further statistical analysis (see text).

**Figure 4.**

Effects of hypercapnia on MEG fields evoked by tone pattern shifts. Root mean square of differential MEG activity evoked by rare tone pattern shifts when compared with control stimuli was averaged across five subjects. **A:** RMS activity was averaged across all sensors at each latency from 100 ms before stimulus onset to 600-ms poststimulus. Note the increased amplitude during the normocapnic when compared with the hypercapnic periods at all latencies. **B:** Activity in each gradiometer channel was averaged between 100 and 500 ms after tone onset. Note the increased amplitude during the normocapnic when compared with the hypercapnic periods at all 204 sensors. The difference between normocapnic and hypercapnic activity was highly significant (see text).

### Hypercapnia attenuates evoked and spontaneous MEG spectral activity

Spectral power was averaged across 20 sensors at the top of the head where contamination of the MEG with muscle activity was not a concern. Estimates of power were calculated for evoked activity in specific frequency bands to novel words in the visual word-processing task from stimulus onset to 600-ms poststimulus. One-way ANOVA showed a significant effect for capnic condition, $F(1,47) = 20.7$, $p < 0.001$. Post hoc $t$-tests identified significantly lower levels of power in the alpha [8–12 Hz; $t(6) =$ 2.1, $p < 0.05$], and beta [9–12 Hz; $t(6) = 2.4$, $p < 0.05$] bands for hypercapnia compared to normocapnia. No such effects were observed for the low [25–55 Hz; $t(6) =$ 0.24, $p > 0.05$] and high [65–95 Hz; $t(6) = 0.7$, $p > 0.05$] gamma bands.

Spectral MEG power was also measured during resting (Figure 5). One-way ANOVA did not reveal a main effect of capnic condition [$F(1,46) = 0.4$, $p = 0.53$]. Hypercapnia attenuated power in all bands (theta by 7%, alpha by 15%, beta by 10%, and gamma by 11%), but post hoc $t$-tests showed that only the gamma decrease was significant [$t(5)$ = 3.9, $p < 0.02$].
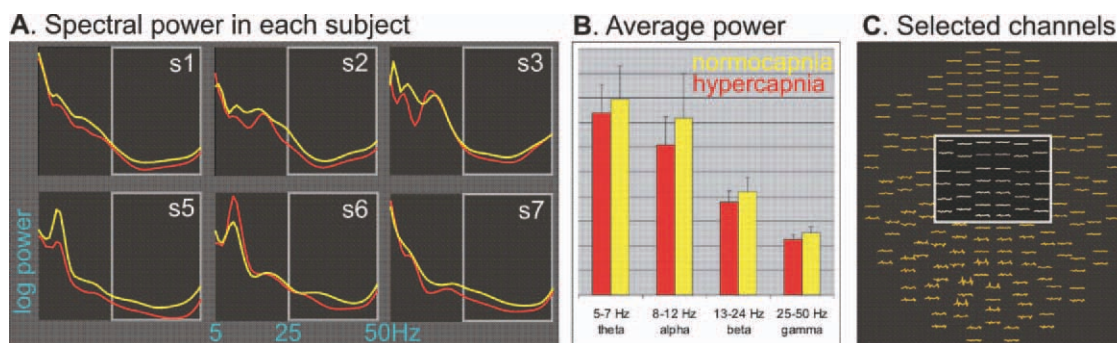


**Figure 5.**

Hypercapnia attenuates spontaneous resting MEG fields. **A:** Spectral power in each of six subjects is plotted against frequency from 5 to 50 Hz. Power is generally greater under normocapnia in most subjects and frequencies, with most variability in the alpha band. During this recording, subjects were resting quietly in a quiet room and may have experienced different levels of alpha activity. The gamma band is indicated by the light gray box. **B:** Power is averaged across subjects for each frequency band, as indicated. Hypercapnia attenuated power in all bands (theta by 7%, alpha by 15%, beta by 10%, and gamma by 11%) but only the gamma decrease was significant (paired $t$-test, two-tailed $P < 0.02$). **C:** Spectral power

## DISCUSSION

Across both auditory and visual modalities, hypercapnia induced significant decreases in ERFs. Amplitude reductions were observed in early sensory components as well as later cognitive components and extended across many cortical regions. Hypercapnia also showed a tendency to decrease spectral power during rest in all frequency bands, an effect that reached significance in the gamma band. These results demonstrate that increased $CO_2$ levels attenuate the event-related cortical neuromagnetic response in the auditory and visual systems during both early sensory as well as later cognitive processing. This effect is prevalent across widespread cortical areas and affects spontaneous brain activity during rest. Neurohemodynamic modeling procedures that assume that neural activity, and thus $CMRO_2$, is constant during hypercapnia may need to be modified to reflect hypercapnia-induced changes in neural activity.

It is unlikely that attentional factors due to hypercapnic breathing can explain the observed effects. The experimental setup minimized any perceived difference between normal and $CO_2$-enriched air, and no subject reported any noticeable difference between conditions. Attention can modulate early sensory components of the evoked potential. However, such effects generally appear in paradigms with rapidly presented competing stimuli. In previous studies, the stimuli showing decreased responses when they are unattended are relatively weak, task-irrelevant stimuli that need to be suppressed in order to efficiently process the task-relevant stimuli [Hillyard and Anllo-Vento, 1998]. This is not the case in the tasks reported here, which measured the responses to strong task-relevant stimuli. If there is any effect of distraction, it might be expected to make the task more difficult, which would tend to increase the size of the N400, whereas the opposite pattern was observed.

### Comparison with Previous Human Studies

Previous examinations of spontaneous EEG spectral power during hypercapnia either combined the low frequency with the high or only looked at power in the low frequencies. Because most EEG power is in the lower frequency bands, most measures that combine all frequencies are insensitive to changes in higher frequencies. Furthermore, the lower frequency bands are often dominated by alpha and other rhythms that are inversely correlated with alertness, arousal, and BOLD [Feige et al., 2005; Goldman et al., 2002]. Thus, low frequency power is problematic as an indicator of activation. In contrast, high gamma power may be a good indicator of neural activation [Logothetis et al., 2001; Miller et al., 2009].

A previous ERP study [Bloch-Salisbury et al., 2000] found no effects of hypercapnia on the N1, P2, or P3 ERP components evoked by a simple auditory signal-detection task. Because MEG sensors have smaller lead fields than EEG, consistent MEG deflections directly comparable to these components cannot be reliably identified. Furthermore, although the generators underlying these ERP components would be expected to also participate in generating the MEG signals evoked by rare tones in our auditory task, they may not be identical [Hari et al., 1982]. When we examined differential RMS power of broad band MEG to rare tone shifts, we found a significant decrease under hypercapnia. Furthermore, we found a significant decrease in the visual sensory response M100 as well as later cognitive responses in the visual word semantic task. Without simultaneous EEG recordings, it is not possible to interpret the differences between our findings and those of Bloch-Salisbury [2000]. However, it is clear from our data that there are reliable decreases in both auditory and visual components at both early and longer latencies involving both sensory and cognitive processing.

It should be noted that possible factors affecting the amplitude, though not the spatial distribution, of EEG and MEG signals may include gray and white-matter anisotropy [Hauseisen et al., 2002] and intracellular and extracellular pH-concentration [Niedermeyer and Lopes da Silva, 1999]. The within-subject design of the current experiment controls for potential anisotropy effects, whereas conductivity changes due to extracellular pH alterations are expected to affect the EEG to a much higher degree than MEG. Intracellular pH, on the other hand, is highly buffered and may show only minimal changes in the current experiment [Deitmer and Chesler, 2008].

### Implications for Calibrated-BOLD Studies

The calibrated-BOLD method was introduced by Davis and colleagues [1998] as a method to use combined measurements of the CBF and BOLD responses to activation to estimate the corresponding fractional change in $CMRO_2$ (%$\Delta CMRO_2$). The method is based on the idea that the BOLD response depends on both the change in CBF and $CMRO_2$, and so by properly combining BOLD and CBF response measurements with an appropriate model for the BOLD response, %$\Delta CMRO_2$ can be estimated. The model for the BOLD signal (fractional signal change $s$) proposed by these authors is as follows:

$$s = M \left(1 - f^{\alpha - \beta} r^{\beta}\right) \tag{1}$$

where $f$ is the ratio of CBF between the active state and the baseline state and $r$ is the ratio of $CMRO_2$ between these two states. The parameter $M$ represents a scaling factor specific to that brain region and depends on baseline state and also on the MRI acquisition (magnetic field strength and echo time). Based on a combination of experimental results and numerical simulations, the parameter $\alpha$ is assumed to be equal to 0.38 [Grubb et al., 1974], while $\beta$ is taken to be 1.5 [Boxerman et al., 1995; Davis et al., 1998]. Although $\alpha$ and $\beta$ are taken to be known parameters, the scaling parameter $M$ must be estimated in each

**TABLE II. Errors in the estimation of activation %$\Delta$CMRO$_2$ with calibrated-BOLD, using recent experimental measurements of CBF and BOLD responses to hypercapnia and activation**

| Study | Brain region | Hypercapnia | | Activation | | Estimated $\Delta$CMRO$_2$ (%)[a] | | %$\Delta$CMRO$_2$ %$\Delta$CBF | |
|---|---|---|---|---|---|---|---|---|---|
| | | $\Delta$CBF (%) | $\Delta$BOLD (%) | $\Delta$CBF (%) | $\Delta$BOLD (%) | $\delta_{CO2} = 0$ | $\delta_{CO2} = -0.11$ | $\delta_{CO2} = 0$ | $\delta_{CO2} = -0.11$ |
| Perthen et al., 2008 | Visual cortex | 48 | 2.6 | 48 | 1.2 | 18.5 | 14.0 | 0.39 | 0.29 |
| Ances et al., 2008 | Visual cortex | 71 | 2.08 | 78 | 0.89 | 32.5 | 28.4 | 0.42 | 0.36 |
| Ances et al., 2008 | Basal ganglia | 42 | 1.52 | 32 | 0.13 | 20.3 | 19.6 | 0.64 | 0.61 |
| Leontiev et al., 2007 | Visual cortex | 66 | 2.52 | 82 | 1.81 | 21.5 | 13.6 | 0.26 | 0.17 |

[a]Calculated from the Davis model [Eq. (1)] with the assumption that the fractional change in CMRO$_2$ with hypercapnia is $\delta_{CO2}$. The standard assumption is $\delta_{CO2} = 0$, and the calculations were repeated assuming that the fractional decrease in CMRO$_2$ is identical to the observed fractional decrease in MEG power in the gamma band ($\delta_{CO2} = -0.11$).

experiment. This is done by measuring CBF and BOLD responses to mild hypercapnia, assuming that hypercapnia increases CBF but does not change CMRO$_2$. With this assumption ($r = 1$ for hypercapnia), $M$ is calculated with Eq. (1) from the measured responses $f$ and $s$ in hypercapnia. This value of $M$ is then combined with the measured responses $f$ and $s$ to a neural stimulus to estimate the CMRO$_2$ ratio $r$ for the neural stimulus. The estimated CMRO$_2$ change with neural activation is often expressed as %$\Delta$CMRO$_2 = 100(r - 1)$.

The assumption that mild hypercapnia does not affect CMRO$_2$ remains controversial [Zappe et al., 2008]. High levels of hypercapnia usually have been found to depress CMRO$_2$ in animal studies [Kliefoth et al., 1979; Sicard and Duong, 2005], although one study found increased CMRO$_2$ after several hours of sustained hypercapnia [Yang and Krasney, 1995]. However, the central question here is whether the mild hypercapnia associated with an awake human subject breathing a 5% CO$_2$ gas mixture also reduces CMRO$_2$. Early human studies using the nitrous oxide method are usually taken to support this assumption [Kety and Schmidt, 1948; Novack et al., 1953]. However, these studies lacked the sensitivity to detect all but the largest changes in CMRO$_2$. Other animal studies [Nilsson and Siesjo, 1976; Sicard and Duong, 2005] support the assumption that mild hypercapnia does not substantially change CMRO$_2$. Nevertheless, the widely divergent conditions of these experiments (species, anaesthesia, level of hypercapnia, and method used to induce hypercapnia) render questionable their significance for small hypercapnic changes in normal behaving humans. For these reasons, the validity of the assumption remains an open question. Conflicting effects of mild hypercapnia on behavioral performance have also been reported, with some reporting no effect on cognitive and psychomotor tasks [Bloch-Salisbury et al., 2000], while others report psychomotor slowing [Fothergill et al., 1991]. As noted earlier, the literature is unclear whether hypercapnia at the levels used for BOLD calibration affects neuronal processing in sensory or association areas of the unanesthetized human cortex. The current results indicate that this is the case.

A useful index of the coupling of flow and metabolism changes is the ratio %$\Delta$CMRO$_2$/%$\Delta$CBF. A number of studies have used the calibrated-BOLD approach to estimate this ratio and have found values in the approximate range 0.2–0.6 [Ances et al., 2008, 2009; Chiarelli et al., 2007a,b; Davis et al., 1998; Hoge et al., 1999; Kastrup et al., 2002; Kim et al., 1999; Leontiev and Buxton, 2007; Leontiev et al., 2007; Perthen et al., 2008; Stefanovic et al., 2004, 2005] (Note that the changes in blood oxygenation that underlie the BOLD effect occur, because this ratio is less than one.) However, these estimates assume that there is no change in CMRO$_2$ with hypercapnia. In the current study, we found that the power in the gamma band of the MEG signal was reduced by 11% when subjects inhaled a 5% CO$_2$ gas mixture. If we assume that CMRO$_2$ decreases by the same amount, we can use the framework of the calibrated-BOLD approach to estimate the size of the error produced in the calculated CMRO$_2$ change or the CMRO$_2$/CBF coupling ratio. For the hypercapnia experiment, we now take $r = 1 + \delta_{CO2}$, where $\delta_{CO2}$ is the fractional change in CMRO$_2$ induced by hypercapnia: in the standard analysis $\delta_{CO2} = 0$, but if CMRO$_2$ decreases by 11%, $\delta_{CO2} = -0.11$. Table II shows the results of calculating %$\Delta$CMRO$_2$ and the CMRO$_2$/CBF coupling ratio for both $\delta_{CO2} = 0$ and $\delta_{CO2} = -0.11$ using mean measured CBF and BOLD responses to activation and hypercapnia from several recent studies [Ances et al., 2008; Leontiev et al., 2007; Perthen et al., 2008] that used the same gas inhalation set-up as the current study. In each case, the neglect of a CMRO$_2$ decrease with hypercapnia leads to an overestimate of %$\Delta$CMRO$_2$ with activation. The CMRO$_2$/CBF coupling ratios, originally estimated to fall in the range 0.26–0.64, are reduced to the range 0.17–0.61 (the reduction varies from 0.03 to 0.1 for the different sets of data). These estimates of the potential errors due to the assumption of constant CMRO$_2$ with mild hypercapnia are similar to previous estimates [Ances et al., 2008].

In summary, if the change in power of the fluctuations of the MEG signal with hypercapnia is an accurate quantitative reflection of the associated change in CMRO$_2$, then studies using the calibrated-BOLD method with

hypercapnic calibration are overestimating the $CMRO_2$ change with activation. However, the errors are modest, with the corrected ratios of the fractional changes in $CMRO_2$ and CBF still falling in the approximate range 0.2–0.6. Recently, an alternative hyperoxia calibration method has been proposed [Chiarelli et al., 2007b], and this method potentially could avoid the errors of the hypercapnia calibration.

### Possible Mechanisms

The MEG is generated by transmembrane currents due to synaptic activity and related active membrane channels [Murakami et al., 2003; Niedermeyer and Lopes da Silva, 1999]. The broad hypercapnia-induced decrease in cortical activity, across different locations, at different latencies, in different sensory modalities as well as cognitive processing, argues for a very general mechanism. Recent work in animals suggests that a mechanism involving pH and adenosine provides a path whereby increased $CO_2$ levels may dampen overall neuronal activity. In rodent hippocampal slices, increasing $CO_2$ from 2 to 5% attenuates the CA1 field EPSP response to stimulation of the Schaffer collaterals by 18% [Dulla et al., 2005], close to the 20% decrease found in the current study. The mechanism of this suppression was shown to involve modulation via pH of presynaptic adenosine A1 receptors, thus decreasing gluatminergic transmitter release. Another potential mechanism is related to temperature. As reviewed by Moore & Cao [2008], hypercapnia-induced changes in vasodilation cause an increase in the rate of arterial blood flow, which, in monkeys, has shown to reduce local brain temperature, which in turn can suppress neuronal activity, consistent with our observations. In either case, hypercapnia would induce a global decrease in synaptic currents and thus link intraneuronal currents, the likely generator of MEG signals.

### Homeostatic Function

The large CBF change associated with hypercapnia increases tissue oxygenation [Tachtsidis et al., 2009], consistent with a large BOLD response reflecting an associated increase of blood oxgenation [Perthen et al., 2008]. For this reason, a shortage of $O_2$ cannot account for the observed changes in neural activity. However, considering that low-cerebral pH could arise due to excessive accumulation of lactate or $CO_2$, this could serve as a signal independent of $O_2$ level itself that CBF is unable to keep pace with neuronal activity. The results observed in the present study may reflect a homeostatic mechanism by which neuronal activity is adjusted to a level that can be sustained by available blood flow. This is consistent with the idea of *oxygen conformance*, the reduction in the demand for $O_2$ as a way to cope with reduced availability of $O_2$ [Hochachka et al., 1996]. Energy use is tightly related to synaptic activity [Attwell and Laughlin, 2001]. By downregulating presynaptic release, the proposed mechanism attenuates the entire cascade of transmitter reuptake and resynthesis as well as postsynaptic depolarization, modulation of intrinsic currents, and consequent cell-firing. Because inhibitory neuronal activation is tightly coupled to excitatory through feedforward and feedback circuits [Buzsáki, 1984], inhibitory cells would also be expected to decrease in a proportional manner.

According to this hypothesis, cerebral acidosis is taken as a sign by the vasculature that neuronal activity is too great to be served by existing blood flow. The well-known increase in CBF is one solution to this problem. The greater blood flow not only restores normal pH, but also other resources that would be expected to be depleted by high activity. We hypothesize that the phenomenon reported here, wherein cerebral acidosis suppresses neuronal activity, serves the same purpose as the increased blood flow. Both seek to correct the imbalance between neuronal activity and blood flow. Note that this hypothesis addresses why acidosis suppresses cortical activity when it is generated by neuronal activity; breathing $CO_2$ reproduces the acidosis and its consequent increase of CBF, but that increase will not correct cortical pH under these artificial circumstances.

The universal nature of the suppression may help explain why, despite its large and widespread effects on neural activity, hypercapnia did not affect performance speed or accuracy on either task. Similar results have previously been obtained with a more extensive neuropsychological battery at moderate levels of hypercapnia that significantly affected spontaneous EEG [Bloch-Salisbury et al., 2000]. It seems plausible that a universal suppression of neurotransmission might provide an optimal strategy for reducing energy use while maintaining function when compared with a selective suppression of limited aspects of neural activity.

## CONCLUSION

We propose that hypercapnia has a multivalent response on both the vasculature and neural activity. This multivalent response can be understood as an adaptive mechanism that interprets lowered pH as a sign that the rCBF is unable to maintain homeostasis, and consequently acts to both decrease neural activity, and increases rCBF in an effort to restore balance. Under this proposal, hypercapnia would also have an indirect effect of reducing rCBF via its effect on neural activity.

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