Impact of Visual Repetition Rate on Intrinsic Properties of Low Frequency Fluctuations in the Visual Network

Yi-Chia Li1,2, Chien-Chung Chen3,4, Jyh-Horng Chen2*

1 Graduate Institute of Biomedical Engineering and Bioinformatics, National Taiwan University, Taipei, Taiwan, 2 Interdisciplinary MRI/MRS Laboratory, Department of Electrical Engineering, National Taiwan University, Taipei, Taiwan, 3 Department of Psychology, National Taiwan University, Taipei, Taiwan, 4 Neurobiology and Cognitive Science Center, National Taiwan University, Taipei, Taiwan

Abstract

Background: Visual processing network is one of the functional networks which have been reliably identified to consistently exist in human resting brains. In our work, we focused on this network and investigated the intrinsic properties of low frequency (0.01–0.08 Hz) fluctuations (LFFs) during changes of visual stimuli. There were two main questions to be discussed in this study: intrinsic properties of LFFs regarding (1) interactions between visual stimuli and resting-state; (2) impact of repetition rate of visual stimuli.

Methodology/Principal Findings: We analyzed scanning sessions that contained rest and visual stimuli in various repetition rates with a novel method. The method included three numerical approaches involving ICA (Independent Component Analyses), fALFF (fractional Amplitude of Low Frequency Fluctuation), and Coherence, to respectively investigate the modulations of visual network pattern, low frequency fluctuation power, and interregional functional connectivity during changes of visual stimuli. We discovered when resting-state was replaced by visual stimuli, more areas were involved in visual processing, and both stronger low frequency fluctuations and higher interregional functional connectivity occurred in visual network. With changes of visual repetition rate, the number of areas which were involved in visual processing, low frequency fluctuation power, and interregional functional connectivity in this network were also modulated.

Conclusions/Significance: To combine the results of prior literatures and our discoveries, intrinsic properties of LFFs in visual network are altered not only by modulations of endogenous factors (eye-open or eye-closed condition; alcohol administration) and disordered behaviors (early blind), but also exogenous sensory stimuli (visual stimuli with various repetition rates). It demonstrates that the intrinsic properties of LFFs are valuable to represent physiological states of human brains.

Introduction

Visual processing network is one of the functional networks which have been reliably identified to consistently exist in human resting brains [1,2]. There have been many studies demonstrating the correlations between endogenous factor/behavior modulations and intrinsic properties of LFFs in this network. Yang et al. discovered significantly higher LFF power of visual cortices in EO (eye-open) than EC (eye-closed) condition during darkness [3]. Esposito et al. observed that alcohol induced resting-state functional connectivity of visual network, which may impair the normal activation response to visual stimuli and affect visual perception [4]. Yu et al. found decreased functional connectivity between primary visual area (PVA) and bilateral supplementary motor area (SMA) in early blind subjects [5]. Summarily, visual network is altered by modulations of both endogenous factors (EO, EC, and alcohol administration) and disordered behaviors (early blind).

In our study, we focused on the correlations between exogenous factor modulations and intrinsic properties of LFFs in visual network. We analyzed scanning sessions that contained rest and visual stimuli in various repetition rates with a novel method. The method included three numerical approaches involving Independent Component Analyses (ICA) [6], fractional Amplitude of Low Frequency Fluctuation (fALFF) [7], and coherence [8], to respectively investigate the modulations of visual network pattern, LFF power, and interregional functional connectivity across resting-state and visual stimuli at different repetition rates. There were two main questions to be discussed in this study: intrinsic properties of LFFs regarding (1) interactions between visual stimuli and resting-state; (2) impact of repetition rate of visual stimuli.

Results

Impact of Visual Repetition Rate on Visual Network Pattern

We performed group ICA-based analyses to obtain visual networks during rest and visual stimulus rate = 2, 4, 8, 16 Hz. In Figure 1, (a)–(e) and (a)′–(e)′ respectively showed lateral and
medial visual areas across various visual repetition rates. The lateral visual areas consisted of peristriate area and lateral and superior occipital gyrus (Brodmann area 19), whereas the medial visual areas predominantly consisted of striate and para striate (Brodmann area 17/18). The two subsystems of visual network fitted in with prior literature [1] which illustrated that the visual network was divided into subsystems by ICA-based analyses. By comparing Fig. 1(b)/(b)' with Fig. 1(a)/(a)', more areas of visual network were involved in visual processing when resting-state was replaced by visual stimuli. With increasing visual repetition rate, more areas of visual network were involved in visual processing, as Fig. 1(b)/(b)'–(d)/(d)' shows. The maximum involved areas occurred in repetition rate = 8 Hz. When repetition rate was even higher, as Fig. 1(e)/(e)' shows, less areas of visual network were involved in visual processing.

Impact of Visual Repetition Rate on LFF power

Figure 2 showed the impact of visual repetition rate on LFF power in primary visual cortex, the dorsal stream, and the ventral stream. When visual repetition rate increased from 0 Hz to 2 Hz, larger fALFF occurred in all these three areas, which indicated that low frequency oscillations of visual network fluctuated more dramatically during visual stimuli rather than resting-state. With higher repetition rate, fALFF in the three areas kept increasing. The largest fALFF occurred in repetition rate = 8 Hz. When repetition rate was even higher, fALFF decreased. This illustrated that low frequency oscillations of visual network fluctuated more dramatically with higher repetition rate. However, the low frequency fluctuations fluctuated the most dramatically in repetition rate = 8 Hz. When repetition rate was even higher, the low frequency oscillations fluctuated less dramatically.

Impact of Visual Repetition Rate on Interregional Functional Connectivity

Figure 3 showed the impact of visual repetition rate on LFF coherence between two visual streams and primary visual cortex. When visual repetition rate increased from 0 Hz to 2 Hz, larger coherence occurred between both two visual streams and primary visual cortex, which represented higher consistency of LFFs between both two streams and primary visual cortex and indicated stronger interregional functional connectivity of visual network during visual stimuli rather than resting-state. With higher visual repetition rate, coherence between both two visual streams and primary visual cortex kept increasing. The largest coherence occurred in repetition rate = 8 Hz. When the repetition rate was even larger, coherence decreased. This showed that higher repetition rate induced stronger interregional functional connectivity in visual network. However, the strongest interregional functional connectivity occurred in repetition rate = 8 Hz. When repetition rate was even higher, weaker inter-regional functional connectivity occurred.

Discussion

Why Did We Choose “Coherence” but not “Correlation” as the Index which Represented Interregional Functional Connectivity?

Compared with correlation analyses, coherence analyses are less sensitive to the shape of regional hemodynamic response function (HRF), which varies across regions due to interregional differences in vascular properties [8]. The insensitivity increases the accuracy of interregional functional connection measures. Therefore, coherence analyses were applied in our study as the index which represented interregional functional connectivity that was invariant to interregional differences in the HRF.

Intrinsic Properties of LFFs Regarding Visual Stimuli at Different Repetition Rates

In our study, we changed visual repetition rate to alter exogenous sensory stimulus conditions. With increasing visual repetition rate, more areas of visual network participated in visual processing, the low frequency oscillations fluctuated more dramatically, and higher interregional functional connectivity occurred. Briefly, stronger interregional functional connections occurred in visual network during visual stimuli rather than resting-state.
between 0–16 Hz. The interregional functional connections were found to peak at 8 Hz. In addition to around 8 Hz peak, previous literatures demonstrated that other peaks were observed for stimulation frequencies at around 20, 40, and 80 Hz [25–27]. These peaks might be due to neural oscillators, which preferably oscillate at certain frequencies, so called “resonance frequencies”.

Figure 1. Impact of repetition rate on the patterns of two subsystems in visual network. (The number in left upper corner of each image represented the location of the image in z-direction of Talairach coordinates.)

doi:10.1371/journal.pone.0018954.g001
In another word, visual stimuli at those resonance frequencies are processed faster by human brains than the other frequencies. Furthermore, smaller BOLD responses to the other frequencies were indicated to be caused by a reduction of neuronal metabolic demand [28], which supported prior demonstrations.

Factors Which Affect Intrinsic Properties of LFFs in Visual Network

Our study focused on the relationship between exogenous factor modulations (various visual repetition rates) and intrinsic properties of LFFs in visual network. According to prior discussions in our work, stronger interregional functional connections occurred in visual network when resting-state was replaced by visual stimuli. With changes of exogenous stimuli (visual repetition rates), interregional functional connections were also modulated. To combine the results of our work with prior literatures [3–5], intrinsic properties of LFFs in visual network are altered by the following factors: endogenous factors (EO and EC condition; alcohol administration), disordered behaviors (early blind), and exogenous sensory stimuli. It demonstrates that
intrinsic properties of LFFs are valuable to represent physiological states of human brains.

**Materials and Methods**

**Ethics Statements**

1. The participant has no history of neurological disorder, medication use, substance abuse, or contraindications to MRI such as claustrophobia.
2. The participant has no metal implants such as a pacemaker or a stent graft.
3. The participant has no make-up or tattoo on the skin.
4. The procedures of this study have been approved by National Taiwan University Hospital, Taipei, Taiwan.
5. The participant understands exactly the procedures of the whole experiment and gives informed written consents to procedures before participation.

**Participants**

Fourteen right-handed volunteers (7 M, 7 F), ranging from 20- to 32-years-old, were recruited, trained, and imaged. Handedness was determined by Edinburgh Handedness Inventory [29]. Score $>40$ was used to ensure right handedness. The mean/std score of the fourteen volunteers was 90/15.19. None of the participants had the history of neurological disorder, medication use, substance abuse, or contraindications to MRI such as claustrophobia. All participants gave informed written consents to procedures approved by Medical Imaging Laboratory Research Ethics Board before participation.

**The Design of Experiment**

Figure 4 (a) showed the design of experiment in our study. There were twelve task periods in the experiment. Each task period involved five stimulus conditions, which were rest and visual stimuli provided with checkerboard twinkling in the repetition rate = 2, 4, 8, and 16 Hz. In the rest condition, subjects were asked to face a screen without any visual stimuli and remain as still as possible, resting with their eyes open.

The five stimulus conditions in a task period were arranged in randomized order to prevent the results of our study from being affected by a regular pattern of stimuli. The duration of each stimulus condition within a task period was 16 s, which could avoid consistent and gradual reduction in activation caused by long duration of repeated visual stimuli (fMR adaptation) [30]. Hence, every task period which included five stimulus conditions lasted 80 s. The total time of the experiment which involved twelve task periods was 16 min. In addition, to avoid the situation that a participant did not concentrate on the visual stimuli during tasks, a tiny colorful cross randomly appeared in the center of the checkerboard. Participants in our study were requested to response by pressing the bell in their right hands to inform us they were still involved in the tasks.

After obtaining scanning sessions that contained rest and visual stimuli in various visual repetition rates, time series of the same stimulus condition in each task period were extracted and combined to become a new time series in a longer duration, as Figure 4 (b) showed. The five new time series of various stimulus conditions were processed in the subsequent analyses.

**The Location Determination of Primary Visual Cortex, Ventral Stream, and Dorsal Stream**

In our study, we focused on three main regions of visual network including primary visual cortex, dorsal stream, and ventral stream and performed regional quantitative analyses to investigate intrinsic properties of LFFs in these regions during visual stimuli at different repetition rates. Particular MRI experiments [31,32] were applied to determine the locations of the three regions in the fourteen participants’ brains.

Dorsal stream served spatial localization in visual system. Figure 5 (a) showed the picture which was shown to the participants in the task for the localization of dorsal stream. The picture consisted of many tiny white spots in the gray background. There were two stimulus conditions in the task, which were the spots freezing in the picture and the spots keeping moving radially.

---

Figure 4. The design of experiment in our study.

doi:10.1371/journal.pone.0018954.g004
back and forth in the picture. Each stimulus condition was 18 s in
duration and appeared alternatively for six times. In the
subsequent GLM (General Linear Model) activation group
analysis, BOLD signals of the two stimulus conditions were
compared to find the location of dorsal stream [33,34], as Figure 5
(b) showed (p<0.001).

Figure 5 (c) and 5 (d) showed some examples of the pictures
which were shown to the participants in the task for the
localization of primary visual cortex and ventral stream. The
upper row in Fig. 5 (c) displayed some examples of the object
pictures whereas the bottom row exhibited some examples of the
scrambled pictures which were formed by arranging the pixels of
the object pictures in scrambled order. The contrast of the
scrambled pictures were the same as that of the object pictures.
Fig. 5 (d) represented a black spot in the center of the background
which was the same as the background of Fig. 5 (c).

There were three stimulus conditions in the task for the
localization of primary visual cortex and ventral stream. In the first
condition, the participants were asked to see the picture shown in
Fig. 5 (d). In the second and third conditions, the object pictures
and the scrambled pictures were respectively shown to the
participants. Each object and scrambled picture appeared for 3 s
in order to avoid visual fatigue to an individual picture. Each
stimulus condition was 18 s in duration and appeared by turns for
time.

Ventral stream and primary visual cortex respectively served
object recognition and receive and transmit visual information in
visual system. In the subsequent GLM activation group analysis,
BOLD signal time series of the scrambled pictures were compared
with those of the object pictures to find the location of ventral
stream [33,34], as Figure 5 (e) showed (p<0.001). In addition,
BOLD signal time series of a black spot in the center were
compared with those of the scrambled pictures to find the location
of primary visual cortex [33,34], as Figure 5 (f) showed (p<0.001).

Acquisition

Scanning was performed on a Bruker Medspec 3.0 Tesla whole
brain body scanner. During the continuous scan, 480 24-slice whole-
brain volumes were acquired using a T2*-weighted gradient-echo,
echo-planar-imaging sequence (TR = 2,000 ms; TE = 30 ms; flip
angle = 84°; slice thickness = 5 mm; slice gap = 0 mm; 4x4 mm
2 in-plane voxel resolution; 64x64 image matrix, FOV
256 x256 mm2). During the scans for the localization of primary
visual cortex, dorsal stream and ventral stream, the same imaging
sequence and parameters were applied. However, 108 whole brain
volumes were acquired in each scan for localizing an individual
region.

At the end of fMRI data collection, fast spin-echo, 24-slice 2D
T1- weighted anatomical images (TR = 2000 ms; TE = 17 ms;
echo train length = 4; slice thickness = 5 mm; slice gap = 0 mm;
1 x 1 mm2 voxel resolution; 256 x256 image matrix) were acquired
in the same slice positions, in order to facilitate the precise
determination of the structures corresponding to the functional
activation foci.

Preprocessing

The five new time series of various stimulus conditions obtained
in Fig. 4 (b) were processed based on the flowchart showed in
Figure 6. GLM activation group analysis and preprocessing which
contained slice timing, rigid body motion correction, spatial
normalization, and Gaussian spatial smoothing (6 mm FWHM
[35]) were performed using the SPM2 tool (http://www.fil.ion.ucl.
a.uk/spm).

Further preprocessing was performed using AFNI (analysis
of functional neuroimaging, http://afni.nimh.nih.gov/ software
[36]. The further preprocessing included linear trend removal,
correction of physiological noises, and temporally band-pass
filtering (BPF; 0.01–0.08 Hz). Linear trend removal reduced
linear drift of BOLD signals which were caused by imperfect
shimming, nonlinearity and instability of gradient field, and loading
effects of radiofrequency (RF) coils [37] during scan. AFNI-based
version of PESTICA (http://www.nitrc.org/projects/pestica/
was used to apply temporal ICA-based analyses to determine
cardiac and respiratory noises from fMRI data without parallel
measurements of physiological signals [38]. PESTICA tool was
also utilized for correcting the determined physiological noises
from the data by RETROICOR (retrospective correction) method
[39], in order to avoid respiratory and cardiac signals to be aliased
into lower frequency band since the BOLD signal was sampled at
a lower frequency (the Nyquist theorem). At the end of further
preprocessing, temporally band-pass filtering (BPF; 0.01–0.08 Hz)
was applied to obtain low frequency fluctuations only. The
preprocessed new time series were applied in subsequent ICA-
based analyses and regional quantitative analyses.

ICA-based Analyses

ICA-based analyses implemented in the GIFT (Group ICA
fMRI Toolbox; http://icat.sourceforge.net/gift/gift_startup.php)
were employed to perform a temporally concatenated group ICA
on the preprocessing new time series to obtain visual networks
during rest and visual stimuli in various repetition rates. We
constrained the number of independent components to 20 [40].
After ICA decomposition we visually inspected all components
and selected functional connectivity maps representing the visual
network. Fig. 1 showed the visual networks during rest and various
visual stimulus conditions.

Regional Quantitative Analyses

In our work, we focused on the three main regions of visual
network including primary visual cortex, dorsal stream and ventral
stream and investigated intrinsic properties of LFFs in these three
regions across different visual stimulus conditions. The numerical
approaches involving fALFF [7] and regional coherence [8] were
applied to help the investigation. Based on the locations of the
three regions which were determined previously, the preprocessed
new time series locating on these regions were selected for regional
quantitative analyses.

In fALFF analyses, we calculated fALFF of the three main
regions across different repetition rates, as Fig. 2 showed. The
results represented the impact of visual repetition rate on low
frequency fluctuation power in the three main regions of vi-
nual network. In coherence analyses, we calculated coherence
of the time series between two visual streams and primary visual
cortex across different repetition rates, as Fig. 3 showed. The results
represented the impact of visual repetition rate on interregional functional connectivity between the two streams and primary visual cortex across different visual stimulus conditions.
Acknowledgments

We thank Instrumentation Center, National Taiwan University for accessing the MRI scanner.

Author Contributions

Conceived and designed the experiments: Y-CL C-CC. Performed the experiments: Y-CL C-CC. Analyzed the data: Y-CL. Contributed reagents/materials/analysis tools: Y-CL J-HC. Wrote the paper: Y-CL.

References

1. Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, et al. (2006) Consistent resting-state networks across healthy subjects. Proc Natl Acad Sci USA 103(37): 13848–13853.
2. Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, et al. (2009) Correspondence of the brain’s functional architecture during activation and rest. Proc Natl Acad Sci USA 106(31): 13040–13045.
3. Yang H, Long XY, Yang Y, Van H, Zhu CZ, et al. (2007) Amplitude of low frequency fluctuation within visual areas revealed by resting-state functional MRI. Neuroimage 36(1): 144–152.
4. Esposito F, Pignataro G, Di Renzo G, Spinali A, Paccone A, et al. (2010) Alcohol increases spontaneous BOLD signal fluctuations in the visual network. Neuroimage 53(2): 534–543.
5. Yu C, Liu Y, Li J, Zhou Y, Wang K, et al. (2008) Altered functional connectivity of primary visual cortex in early blindness. Hum Brain Mapp 29(5): 533–543.
6. Calhoun VD, Adali T, Pearlson GD, Pekar JJ (2001) A method for making group inferences from functional MRI data using independent component analysis. Hum Brain Mapp 14(3): 140–151.
7. Zou QH, Zhu CZ, Yang Y, Zuo XN, Long XY, et al. (2008) An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. J Neurosci Methods 172(1): 137–142.
8. Yu C, Liu Y, Li J, Zhou Y, Wang K, et al. (2008) Altered functional connectivity of primary visual cortex in early blindness. Hum Brain Mapp 29(5): 533–543.
9. Kwong KK, Belliveau JW, Chesler DA, Goldberg IE, Weisskoff RM, et al. (1992) Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. Proc Natl Acad Sci USA 89(12): 5675–5679.
10. Fox MD, Raichle ME (1994) Stimulus rate dependence of regional cerebral blood flow in human striate cortex, demonstrated by positron emission tomography. J Neurophysiol 61(5): 1109–1120.
11. Anderson SJ, Holliday IE, Singh KD, Harding GF (1996) Localization and functional analysis of human cortical area V5 using magnetoencephalography. Proc Biol Sci 263(1369): 423–431.
12. Fylan F, Holliday IE, Singh KD, Anderson SJ, Harding GF (1997) Magnetoencephalographic investigation of human cortical area V1 using color stimuli. Neuroimage 6(1): 47–57.
13. Herrmann CS (2001) Human EEG responses to 1–100 Hz flicker: resonance phenomena in visual cortex and their potential correlation to cognitive phenomena. Exp Brain Res 137: 346–353.

Stimulus Rate Dependence of LFFs in Visual Network

Figure 6. The flowchart of image processing in our study. doi:10.1371/journal.pone.0018954.g006

Author Contributions

Conceived and designed the experiments: Y-CL C-CC. Performed the experiments: Y-CL C-CC. Analyzed the data: Y-CL. Contributed reagents/materials/analysis tools: Y-CL J-HC. Wrote the paper: Y-CL.
26. Emir UE, Bayraktaroglu Z, Ozturk C, Ademoglu A, Demiralp T (2008) Changes in BOLD transients with visual stimuli across 1–44 Hz. Neurosci Lett 436(2): 185–188.
27. Pastor MA, Artieda J, Arbizu J, Valencia M, Masdeu JC (2003) Human cerebral activation during steady-state visual-evoked responses. J Neurosci 23(37): 11621–11627.
28. Parkes LM, Fries P, Knoskens CM, Norris DG (2004) Reduced BOLD response to periodic visual stimulation. Neuroimage 21(1): 236–243.
29. Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9(1): 97–113.
30. Kourtzi Z, Grill-Spector K (2005) fMRI adaptation: a tool for studying visual representations in the primate brain. In: Clifford CWG, Rhodes G, eds. Fitting the mind to the world: Adaptation and after-effects in high level vision. New York: Oxford University Press. ch 6.
31. Huk AC, Dougherty RF, Heeger DJ (2002) Retinotopy and functional subdivision of human areas MT and MST. J Neurosci 22(16): 7195–7205.
32. Malach R, Reppas JB, Benson RR, Kwong KK, Jiang H, et al. (1995) Object-related activity revealed by functional magnetic resonance imaging in human occipital cortex. Proc Natl Acad Sci USA 92(18): 8135–8139.
33. Schneider GE (1969) Two visual systems. Science 163: 895–902.
34. Ungerleider LG, Mishkin M (1982) Two cortical visual systems. In: Ingle DJ, Goodale MA, Mansfield RJW, eds. Analysis of visual behavior. Cambridge, MA: MIT Press. 549 p.
35. Chu C, Ni Y, Tan G, Saunders C, Ashburner J (2010) Kernel regression for fMRI pattern prediction. Neuroimage 56(2): 662–673.
36. Cox RW (1996) AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res 29(3): 162–173.
37. Huettel SA, Song AW, McCarthy G (2009) Functional magnetic resonance imaging. Sunderland Mass.: Sinauer Associates. 258 p.
38. Beall EB, Lowe MJ (2007) Isolating physiologic noise sources with independently determined spatial measures. Neuroimage 37(4): 1286–1300.
39. Glover GH, Li TQ, Rees D (2000) Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. Magn Reson Med 44(1): 162–167.
40. Schöpf V, Windischberger C, Kasses CH, Lanzenberger R, Moser E. (2010) Group ICA of resting-state data: a comparison. MAGMA 23(5–6): 317–325.