Age differences in thalamic low-frequency fluctuations
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The thalamus plays a role in many different types of cognitive processes and is critical for communication between disparate cortical regions. Given its critical role in coordinating cognitive processes, it is important to understand how its function might be affected by aging. In the present study, we examined whether there are age differences in low-frequency fluctuations during rest in the thalamus. Across independent data sets, we found that the amplitude of low-frequency (0.01–0.10 Hz) oscillations was greater in the thalamus among older than younger adults. Breaking this low-frequency range down further revealed that this increase in amplitude with age in the thalamus was most pronounced at the low end of the frequency range (0.010–0.027 Hz), whereas in the higher low-frequency range (0.198–0.250 Hz) younger adults showed greater amplitude than older adults. These shifts in thalamic low-frequency oscillatory activity likely influence the complex dynamics of coordinated brain activity and influence cognitive performance. *NeuroReport* 24:349–353 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Attempts at understanding the brain mechanisms underlying age-related cognitive processing declines have focused on declines in cortical regions. Less attention has been paid to subcortical regions such as the thalamus. Yet, the thalamus plays a central role in cognition; nearly all information into the cortex passes through the thalamus. Furthermore, the thalamus helps to coordinate activity among distant cortical regions.

The thalamus decreases in volume with age, with a linear course of change [1–3] that is associated with diminished cognitive speed [4], and thalamic white matter integrity also declines with age [5–8]. Despite the thalamic structural declines in aging and their association with cognitive speed, functional MRI (fMRI) studies have found few age differences in overall thalamic activation levels during cognitive tasks [9]. However, one question not addressed by previous neuroimaging research contrasting activity during different cognitive tasks is whether thalamic oscillatory activity changes with age. Oscillatory behavior within the brain provides a mechanism for synchronizing activity across disparate brain regions [10].

In the current study, with two independent data sets, we examined how amplitudes of low-frequency oscillations in the thalamus differ with age using as a measure the fractional amplitude of low-frequency fluctuations (fALFF) [11]. This measure is the power within a specific frequency range divided by the total power in the entire detectable frequency range. Thus, fALFF provides a normalized measure of specific low-frequency oscillations relative to the rest of the available range. Its sensitivity and specificity to detect spontaneous brain activity is better than the nonnormalized ALFF measure [11].

Methods
Participants and data acquisition
University of Southern California

Twenty younger (12 males, $M_{age} = 25.35$, age range 19–37) and twenty older adults (10 males, $M_{age} = 68.10$, age range 61–78) participated after screening for right handedness and medical, neurological, and psychiatric disorders. All participants provided written informed consent approved by the University of Southern California (USC) Institutional Review Board. Imaging data were collected at the USC Dana and David Dornsife Neuroimaging Center with a 3T Siemens MAGNETOM Trio scanner (Siemens, Erlangen, Germany) and a 12-channel matrix head coil. The resting-state fMRI data were acquired with the following parameters: repetition time (TR) = 2000 ms, echo time (TE) = 25 ms, slice thickness = 3 mm, interslice gap = 0 mm, flip angle (FA) = 90°, and field of view (FOV) = 192 mm × 192 mm. During the 312 s of scanning, participants were instructed to close their eyes and relax, but not to fall asleep. A high-resolution structural image was also obtained from every participant.

International Consortium on Brain Mapping

Twenty-one younger (seven males, $M_{age} = 22.71$, age range 19–31) and twenty-one older adults (10 males, $M_{age} = 66.81$, age range 56–85) were obtained from a publicly available data set of 86 participants' resting-state fMRI and structural scans [12]. To match the USC data set in sample size and age range, scans from participants between the ages of 32 and 55 were excluded. In addition, scans that did not have full brain coverage were discarded, leaving two 256-s resting-state fMRI scans and one anatomical scan per person included in the analyses.

Data preprocessing

Resting-state fMRI scans were processed using scripts released by the 1000 Functional Connectomes Project [13]. Data preprocessing steps included: (a) three-dimensional motion correction, (b) skull stripping, (c) time series despiking, (d) removal of linear trends, (e) spatial smoothing with a 6-mm full-width half-maximum Gaussian kernel, and (f) mean-based intensity normalization by scaling all volumes by a factor of 10 000. An estimate of the linear transformation (12 degrees of freedom) from individual functional space to the standard space (Montreal Neurological Institute's 152-brain template; isotropic 2-mm voxel size) was also conducted for every resting-state scan using FSL FLIRT [14].

Fractional amplitude of low-frequency fluctuation

For amplitude measures at each voxel we used the fast Fourier transform-based fALFF, as implemented by Biswal *et al.* [13]. Using FSL commands, we first transformed the time series of each voxel to the frequency domain and obtained the power spectrum. The low-frequency band (0.01–0.10 Hz) was then extracted from the power spectrum. At every voxel, the amplitudes across the low-frequency band were summed. Next, fALFF was calculated by dividing the value in each voxel by the sum of amplitudes across the whole frequency domain. Across the whole brain, these values yielded three-dimensional fALFF maps for each functional scan. For every functional scan, these three-dimensional maps were normalized to Z-scores and transformed into the standard MNI152 2 mm^3 space. Age comparisons were conducted with group-level analyses using flameo (FSL) and the Z-score fALFF maps in standard space. Lastly, easythresh (FSL) was applied for multiple comparisons correction ($Z > 2.3$, corrected cluster significance: $P < 0.05$).

Fractional amplitude of low-frequency fluctuation in specific frequency bands

On the basis of categorizations used in previous research [15,16], we subdivided the frequency range into the following bands: slow5 (0.010–0.027 Hz), slow4 (0.027–0.073 Hz), slow3 (0.073–0.198 Hz), and slow2 (0.198–0.250 Hz; the high frequency bound was constrained by the scan repetition time). With the exception of which frequency range was allowed through the band-pass filter, the procedures for obtaining the five slow fALFF Z-score maps followed that of the initial fALFF analysis covering the 0.01–0.10 Hz range.

We conducted whole-brain contrasts of younger and older adults' fALFF for each of these frequency bands. To summarize the results of these five whole-brain contrasts, we used frequency counts of the voxels, for which younger adults showed significantly higher fALFF than older adults, and vice versa, for each frequency band within standard regions of interest (ROIs) of the thalamus. Group and frequency differences in thalamus

fALFF were also examined within individually defined thalamus ROIs.

Region of interest delineation

Structurally defined standard masks of the thalamus were used for the frequency voxel counts. These standard ROIs of the thalamus were defined by the Harvard-Oxford atlas's (*http://www.cma.mgh.harvard.edu/fsl_atlas.html*) probabilistic thalamus map at a 50% probability threshold.

Individually defined thalamus ROIs were created by first skull stripping every anatomical brain using FSL BET (Brain Extraction Tool) [17]. Bilateral thalami were then segmented from each participant's high-resolution structural scan using FSL FIRST [18]. Postsegmentation, manual correction of thalamus masks involved removing erroneous voxels in nonthalamus regions such as ventricles, hippocampus, and brain stem [19]. The final thalamus masks were transformed into standard MNI152 2 mm^3 space.

Results
Thalamus structural volume

Volumes of the individually segmented thalami were compared in a 2 (age: younger, older) × 2 (side: right, left) repeated-measures analysis of variance. In both data sets, younger adults had significantly greater thalamic volume than older adults; for the USC group [older, $M = 8908.4$ mm^3; younger, $M = 10235.2$ mm^3; $F(1,38) = 22.12$, $P < 0.001$, $\eta^2_p = 0.37$]; for the International Consortium on Brain Mapping (ICBM) group [older, $M = 8895.2$ mm^3; younger, $M = 10202.3$ mm^3; $F(1,40) = 30.52$, $P < 0.001$, $\eta^2_p = 0.43$]. Consistent with previous findings [20], in both data sets, there also was significantly greater left than right thalamic volume; USC [right, $M = 9434.8$ mm^3; left, $M = 9708.8$ mm^3; $F(1,38) = 17.62$, $P < 0.001$, $\eta^2_p = 0.32$]; ICBM [right, $M = 9393.5$ mm^3; left, $M = 9704.0$ mm^3; $F(1,40) = 42.10$, $P < 0.001$, $\eta^2_p = 0.51$]. In neither data set did age and side interact significantly.

Fractional amplitude of low-frequency fluctuation in whole-brain contrasts

Across both data sets we analyzed, whole-brain analyses revealed greater fALFF values (0.01–0.10 Hz) in the thalamus for older adults than for younger adults (Fig. 1).
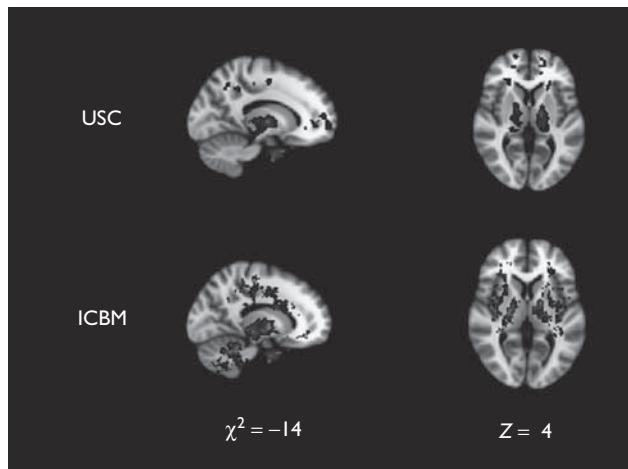
Fractional amplitude of low-frequency fluctuation in specific frequency bands for whole-brain contrasts

We conducted whole-brain contrasts of younger and older adults' fALFF separately for four separate frequency bands [0.010–0.027 Hz (slow5), 0.027–0.073 Hz (slow4), 0.073–0.198 Hz (slow3), and 0.198–0.250 Hz (slow2)]. Then we used the bilateral atlas-based thalamus ROIs to conduct frequency counts of the number of thalamus voxels that showed significant age differences in fALFF. In the slow5 frequency band, 340 voxels had significantly

higher fALFF for older than for younger adults and none showed significantly higher fALFF for younger than for older adults (Fig. 2a). However, this age difference favoring older adults was only seen in this lowest frequency band, and actually reversed in the higher frequency bands, such that younger adults showed higher fALFF than older adults. A χ^2-test of these frequency counts revealed that their distribution across frequency bands differed by age group ($\chi^2 = 602.00$, $P < 0.001$). The same pattern was found in the ICBM data, as well ($\chi^2 = 1079.81$, $P < 0.001$; Fig. 2b).

Individual fractional amplitude of low-frequency fluctuation averages within structural region of interests

The previous analyses compared fALFF values in each voxel across participants, using a standard atlas-defined thalamus ROI. As older adults showed structural decline in the thalamus, we checked if the results would also be evident when using individually delineated ROIs. Thus, in a secondary analysis, we computed the fALFF for each frequency band averaged across the whole right and left thalamus ROI for each participant, using the thalamus ROIs delineated separately for each individual. We then compared these average fALFF values for younger and older participants. In both data sets, in the slow5 frequency band, older adults had significantly greater fALFF than younger adults (Table 1). In addition, in the ICBM data set, older adults had significantly lower fALFF in the slow2 frequency band. The middle
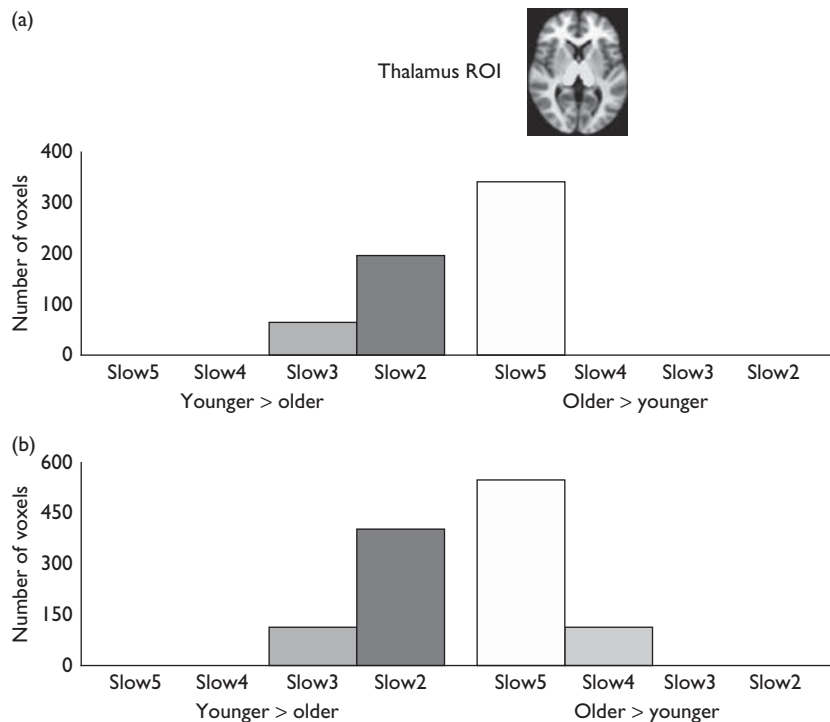
Fig. 1

In both data sets (USC and ICBM), the fractional amplitude of low-frequency fluctuations (the ratio of 00.01−0.1 Hz to the entire frequency range) was significantly greater in older than younger adults in the thalamus. ICBM, International Consortium on Brain Mapping; USC, University of Southern California.

Fig. 2

Number of voxels that showed significant age differences in whole-brain comparisons of fALFF by frequency band for data from (a) USC and (b) ICBM. fALFF, fractional amplitude of low-frequency fluctuations; ICBM, International Consortium on Brain Mapping; ROI, region of interest; USC, University of Southern California.

Table 1 Average fractional amplitude of low-frequency fluctuations in individually delineated right and left thalamus regions of interest for each frequency band for younger and older adults

	Left thalamus		Right thalamus	
Frequency bands	Younger	Older	Younger	Older
USC data				
Slow5	− 0.312	− 0.201*	− 0.308	− 0.207*
Slow4	0.023	0.051	0.054	0.064
Slow3	0.237	0.165	0.223	0.179
Slow2	0.046	0.002	0.047	− 0.017
ICBM data				
Slow5	− 1.044	− 0.505*	− 1.016	− 0.575*
Slow4	0.141	0.456	0.186	0.371
Slow3	0.613	0.600	0.594	0.600
Slow2	− 0.003*	− 0.601	− 0.157*	− 0.633

fALFF, fractional amplitude of low-frequency fluctuations; ICBM, International Consortium on Brain Mapping; USC, University of Southern California.
*Significantly higher fALFF values than the other age group, $P < 0.05$.

frequency bands showed no significant age differences. Thus, consistent with the whole-brain analyses, older adults showed greater fALFF than younger adults in the lower frequency band but not in higher frequency bands.

In addition, we checked whether the structural volume of the thalamus was associated with fALFF values. When controlling for age, there were no significant correlations between thalamus volume (either right or left) and the fALFF of the four frequency bands (of either the right or left thalamus). Thus, the age-related changes in fALFF were not accounted for by structural volume declines.

Discussion
In two independent data sets we found that low-frequency (0.01–0.10 Hz) oscillations had greater amplitudes in older adults than in younger adults in the thalamus. When we examined the amplitude of oscillations separately for different frequency bands, a shift was evident across age within the thalamus, such that older adults had greater amplitudes than younger adults at the slowest frequency band categorization (the 'slow5' band from 0.010 to 0.027 Hz), whereas younger adults had greater amplitudes than older adults at the fastest frequency band categorization (the 'slow2' band from 0.198 to 0.250 Hz).

Low-frequency oscillations have been shown in many studies to reveal functional brain networks [21–23]. The age differences we found in low-frequency oscillations in the thalamus, suggest that older (compared to younger) adults will also show changes in functional connectivity with the thalamus. If so, these changes could have a broad impact on cognition, memory, and behavior, as the thalamus plays a key role in much of the communication between different cortical regions [24].

Thus, questioning how functional connectivity with the thalamus might change with age is an important next question for this line of research. A typical approach for finding functional connectivity of an ROI is to aggregate

the pattern of activity in that region and correlate the timeline of that activity with every other voxel in the brain. As can be seen in Fig. 1, the age differences in low-frequency oscillations were evident in most voxels within the thalamus. However, as the thalamus comprises clearly delineated subregions that have distinct connections with different cortical regions, it will be important that future studies use approaches that do not assume the whole thalamus has similar patterns of functional connectivity.

Oscillatory behavior not only supports important functions including biasing input selection and replaying recent inputs to consolidate memory, but it also provides a mechanism for synchronizing diverse regional activities in the brain. When studying coordination of brain activity across different regions, a key region to inspect is the thalamus; the thalamus is also responsible for processing new perceptual inputs. Thus, our characterization of how oscillatory dynamics within the thalamus change with age is especially important for understanding age-related change in coordinated activity across disparate brain regions. The current findings reveal age-related changes in low-frequency fluctuations in the thalamus. Future work should fully investigate potential age-related changes in oscillatory activity (by looking at both higher and lower frequencies) through simultaneous EEG and fMRI recordings of younger and older adults during resting state.

Conclusion
This study revealed greater amplitude low-frequency (0.01–0.10 Hz) fluctuations in the thalamus among older than among younger adults and these age differences were most pronounced at the slowest frequency band categorization examined (the 'slow5' band from 0.010 to 0.027 Hz).

Acknowledgements

Conflicts of interest
There are no conflicts of interest.

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