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New hypothesis on pontine-frontal eye field connectivity in Kleine-Levin syndrome

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Summary

Previous studies have indicated involvement of the thalamus and the pons in the Kleine-Levin syndrome. In the present study, we investigated functional connectivity of the thalamus and the pons in asymptomatic Kleine-Levin syndrome patients and healthy controls. Twelve patients and 14 healthy controls were investigated by functional magnetic resonance imaging during rest. Resting state images were analysed using seed regions of interest in the thalamus and the pons. Results showed significantly lower functional connectivity between the pons and the frontal eye field in persons with Kleine-Levin syndrome compared to healthy controls. There were no connectivity differences involving the thalamus. Based on these findings, we propose a relation between the sleep disorder Kleine-Levin syndrome and cerebral control of eye movements, which in turn is related to visual attention and working memory. This hypothesis has to be tested in future studies of oculomotor control in the Kleine-Levin syndrome.

1. Introduction

The Kleine-Levin syndrome (KLS) or periodic idiopathic hypersomnia is characterized by sleep episodes that can endure up to several weeks and recur several times a year. During the hypersomnic periods, KLS patients often suffer from behavioural, perceptual, and cognitive disturbances, such as binge eating, delusions, and memory problems (Arnulf *et al.*, 2005). The neuropathology of KLS is unknown. Structural neuroimaging and inter-episodic EEG are usually normal. However, functional neuroimaging (Engström *et al.*, 2009; Engström *et al.*, 2013; Dauvilliers *et al.*, 2014; Hong *et al.*, 2006; Huang *et al.*, 2005) and a few post mortem studies (Arnulf *et al.*, 2005) indicate frontotemporal dysfunction and possible thalamic pathology. In functional magnetic resonance imaging (fMRI) studies, we have previously shown that KLS patients have thalamic hyperactivation when challenged with a working memory task (Engström *et al.*, 2009; Engström *et al.*, 2013). We also showed that KLS patients had lower working memory performance than healthy controls. A later study showed that thalamic hyperactivity in KLS could be a secondary compensatory effect suggesting that the thalamus might not be a primary focus of KLS neuropathology (Engström *et al.*, 2014a).

Recently, we showed that resting state thalamic connectivity appeared normal in one KLS patient during an asymptomatic period (Engström *et al.*, 2014b). However, during hypersomnia the connectivity between the thalamus and the pons was substantially reduced. An interesting observation was that this particular patient, in contrast to the other KLS patients, had congenital horizontal nystagmus, indicating possible influence from the medulla or the dorsal pontine reticular formation. We hypothesized that this particular patient's nystagmus and hypersomnia might have their pathological origin in adjacent dorsal pontine regions.

Here, we investigated if dorsal pons connectivity is abnormal in a group of KLS patients also during asymptomatic periods. If we would find abnormal pontine connectivity in asymptomatic KLS patients, the dorsal pons might be a key network node in KLS. Based on our previous study (Engström *et al.*, 2014b), we expected to find normal resting state

thalamic connectivity in KLS. The aim of the present study is to further explore the elusive neuropathology of KLS by investigating pontine and thalamic functional connectivity during rest in a group of KLS patients and matched healthy controls.

2. Methods

3.1. Participants

Twelve patients diagnosed with KLS according to the International classification of sleep disorders (American Academy of Sleep Medicine, 2005) were included in the study. Four patients were males and eight females, and the patients' mean age was 23.8 years (sd = 9.1 years). Descriptive statistics of the patients regarding sleep episodes is found in table 1. All KLS patients were asymptomatic at the time of the study. One of the patients had congenital nystagmus as reported in a previous study by us (Engström *et al.*, 2014b), the other patients had not been investigated for and had not reported any oculomotor deficits. We made the data analysis both with and without the patient with nystagmus. We also investigated fourteen healthy controls (seven females). The mean age of the controls was 24.3 years (sd=6.9 years). The control group was matched with respect to gender and age to the KLS group. The Regional Ethical Review Board in Linköping, Sweden, approved the study. Oral informed consent was obtained from all participants.

Table 1. Demographic data of Kleine-Levine syndrome (KLS) patients. The table shows mean, standard deviation (sd), median, minimum (min), maximum (max), and range for age at KLS onset, number (#) of KLS episodes during the previous 2 years, total number of episodes, duration of episodes estimated as median, minimum and maximum number of days in each patient, and symptom frequency counted as days/year.

	Age at onset	# Episodes prev. 2 years	Total # episodes	Duration episodes, median	Duration episodes, min	Duration episodes, max	Symptom frequency, days
Mean	14.1	7.2	23.0	14.1	7.7	31.9	82.9
Sd	2.2	4.5	17.2	8.1	4.0	30.1	55.3
Median	15	7	19	14	7	21	64.2
Min	9	1	4	3	3	10	19.0
Max	17	14	57	30	14	104	204.0
Range	8	13	53	27	11	94	185.0

3.2. Image acquisition and analysis

Resting state connectivity was investigated by blood oxygen level dependent (BOLD) fMRI on a Philips Achieva 1.5 T scanner. The following imaging parameters were used: echo time, TE = 40 ms, repetition time, TR = 2700 ms, flip angle = 90°, matrix = 80×80, number of slices = 32, voxel size = 3×3×3 mm^3, no slice gap, scanner mode = interleaved, number of dynamics = 80. Total scan time was 216 seconds. The participants were instructed to rest with their eyes closed during the examination.

BOLD fMRI images were preprocessed using SPM8 software (Wellcome Department of

Imaging Neuroscience, University College, London, UK). Images were realigned to correct for movement during scanning and normalized to standard stereotactic space in Montreal Neurological Institute (MNI) co-ordinates. The normalized images were smoothed with a full width half maximum (FWHM) Gaussian kernel of 8 mm to reduce noise and to compensate for individual anatomical differences in the subsequent voxel-based image analysis.

Seed-based functional connectivity was calculated using linear correlations between time series, employing the RESTing state fMRI analysis toolkit (Song *et al.*, 2011). Seed selection was based on previous studies with independent data showing thalamic hyperactivation and abnormal pontine connectivity (Engström *et al.*, 2014a; Engström *et al.*, 2014b). The seeds consisted of spheres with radii = 8 mm placed in the left thalamus [-10, -4, 6] and the dorsal pons [-6, -34, -24]. The spherical seed regions were modified in order to contain only brain tissue representing the thalamus and the pons. Therefore, image voxels representing cerebrospinal fluid (CSF) were removed from the spherical seed regions. Linear trends in data were removed and the images were band pass filtered in the range 0.01–0.1 Hz. Global signal and movement parameters were not employed as nuisance regressors.

For each participant, the mean time courses within the thalamic and pontine seeds were correlated to the average time courses in all image voxels of the whole brain. The individual correlation maps were normalized using Fisher's Z transform. The Z-maps of all participants were entered two-sample t-tests to compare functional connectivity between KLS patients and healthy controls.

3. Results and discussion

Based on our previous results (Engström *et al.*, 2014b), we expected normal thalamic connectivity during the asymptomatic period, which also was confirmed, since we did not observe any differences between KLS patients and controls when comparing resting state connectivity from the thalamus seed.

On the other hand, we observed a significant difference in resting state pontine connectivity when comparing KLS patients and healthy controls. As shown in Figure 1, KLS patients had significantly lower connectivity between the left dorsal pons and the right frontal eye field, p = 0.041 corrected for multiple comparison at the whole brain level using family wise error (FWE) correction. The peak of the connectivity difference was located at MNI co-ordinates [20, -6, 62], which is adjacent to the peak of antisaccade activation in the frontal eye field identified from a recent meta-analysis (Jamadar *et al.*, 2013). Excluding the unique case of KLS with nystagmus showed similar results but with higher significance, p = 0.027, for the connectivity between the dorsal pons and the frontal eye fields. No other regions were significantly connected to the dorsal pons seed region.
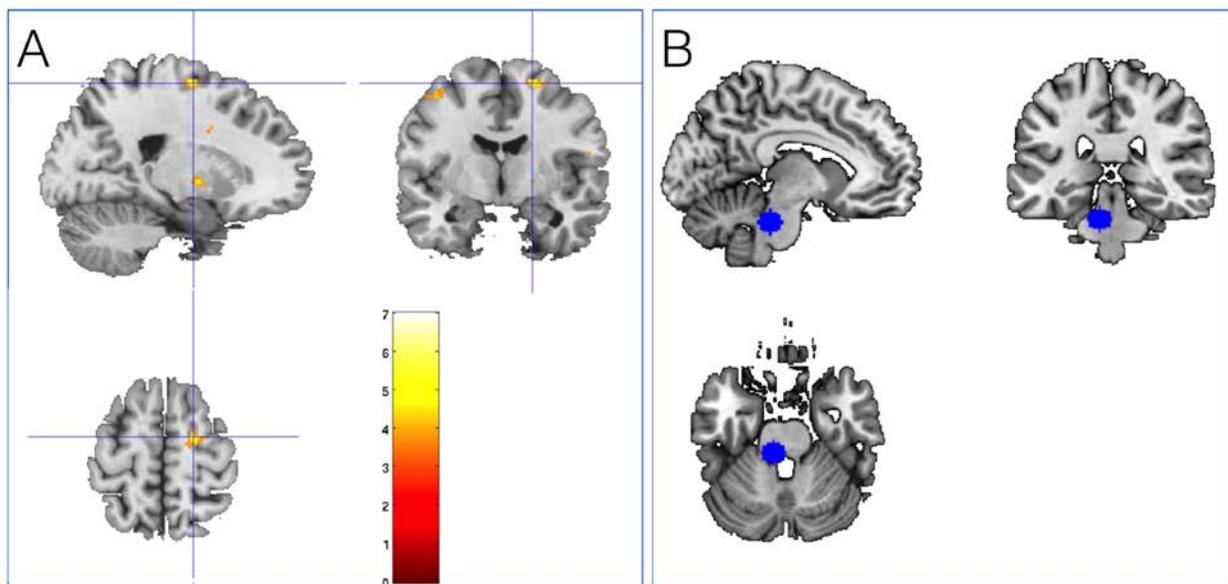

Figure 1. Lower pontine – frontal eye field connectivity in Kleine-Levin syndrome (KLS) patients. The figure shows that KLS patients had significantly lower connectivity between the left dorsal pons and the right frontal eye field. The cross hair is located at the voxel with most significantly different connectivity between healthy controls and KLS patients. The colour bar indicates the T-statistic values. Images were thresholded at uncorrected p-value of 0.001. Peak p = 0.041, corrected for multiple comparisons at the whole brain level.

In the current study, we found reduced connectivity between the dorsal pons and the frontal eye field in a group of asymptomatic KLS patients with previously reported working memory deficits (Landtblom *et al.*, 2003; Engström *et al.*, 2009; Engström *et al.*, 2013). Adjacent pontine nuclei are involved in both regulation of sleep and wakefulness and oculomotor control (Lynch *et al.*, 2006). The frontal eye field controls eye movements such as saccades and antisaccades (Lynch *et al.*, 2006; Jamadar *et al.*, 2013). There are strong connections between the frontal eye field and the brain stem oculomotor system, including the pontine reticular formation, and the basal ganglia, *i.e.* the caudate and the putamen (Lynch *et al.*, 2006). Thus, functional connectivity findings from resting state fMRI data in the current study are in line with well-known cortical-subcortical connections related to the control of eye movements. Interestingly for our results, in subjects with normal vision, there is a relation between the frontal eye field and visual attention processes, which are closely associated with working memory function (Corbetta *et al.*, 1998; Eimer, 2014; Clough *et al.,* 2015). That is to say, there is a cortical network including the frontal eye field and temporoparietal regions that is recruited during voluntary shifts of attention and saccadic eye movements independently of oculomotor deficits (Corbetta *et al.*, 1998).

In a previous study, we fond reduced connectivity between the thalamus and the dorsal pons in a hypersomnic KLS patient with congenital nystagmus (Engström *et al.*, 2014b). We are well aware that the findings of aberrant thalamo-pontine connectivity in one KLS patient with congenital nystagmus may be coincidental. However, the findings of abnormal functional connectivity involving the frontal eye field in a rather large group of KLS patients is of great interest although these observations might be independent of each other. Investigating pontine connectivity must also be performed with caution since fMRI images from the brain stem are prone to cardiac, respiratory, and other artefacts.

In conclusion, we propose, as a new hypothesis, that there might be a connection between areas of the brain controlling sleep, eye movements, and cognitive function in KLS. Future studies using for example eye tracking and saccade-antisaccade paradigms in addition to high resolution MRI for brain stem nuclei detection is needed to verify this hypothesis

Acknowledgements

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