Low Thalamic NAA-Concentration Corresponds to Strong Neural Activation in Working Memory in Kleine-Levin Syndrome

Patrick Vigren1,2,3, Anders Tisell2,3,9, Maria Engström2,4, Thomas Karlsson2,5, Olof Leinhard Dahlqvist2,3, Peter Lundberg2,6,7, Anne-Marie Landtblom2,8

1 Department of Clinical and Experimental Medicine (IKE)/Neuroscience, Linköping University, and Department of Neurosurgery, County Council of Östergötland, Linköping, Sweden, 2 Center of Medical Image Science and Visualization (CMIV), Linköping University, Linköping, Sweden, 3 Radiation Physics, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden, 4 Radiology, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden, 5 Department of Behavioural Science and Learning, Linköping University, Linköping, Sweden, 6 Radiation Physics, Department of Medical and Health Sciences, Linköping University, and Department of Radiation Physics UHL, County Council of Östergötland, Linköping, Sweden, 7 Radiology, Department of Medical and Health Sciences, Linköping University, and Department of Radiology UHL, County Council of Östergötland, Linköping, Sweden, 8 Department of Clinical and Experimental Medicine (IKE)/Neuroscience, Linköping University, and Department of Neurology, County Council of Östergötland, Linköping, Sweden

Abstract

Background: Kleine Levin Syndrome (KLS) is a rare disorder of periodic hypersomnia and behavioural disturbances in young individuals. It has previously been shown to be associated with disturbances of working memory (WM), which, in turn, was associated with higher activation of the thalamus with increasing WM load, demonstrated with functional magnetic resonance imaging (fMRI). In this study we aimed to further elucidate how these findings are related to the metabolism of the thalamus.

Methods: fMRI and magnetic resonance spectroscopy were applied while performing a WM task. Standard metabolites were examined: n-acetylaspartate (NAA), myo-inositol, choline, creatine and glutamate-glutamine. Fourteen KLS-patients and 15 healthy controls participated in the study. The patients with active disease were examined in asymptomatic periods.

Results: There was a statistically significant negative correlation between thalamic fMRI-activation and thalamic NAA, i.e., high fMRI-activation corresponded to low NAA-levels. This correlation was not seen in healthy controls. Thalamic levels of NAA in patients and controls showed no significant differences between the groups. None of the other metabolites showed any co-variation with fMRI-activation.

Conclusion: This study shows negative correlation between NAA-levels and fMRI-activity in the left thalamus of KLS-patients while performing a WM task. This correlation could not be found in healthy control subjects, primarily interpreted as an effect of increased effort in the patient group upon performing the task. It might indicate a disturbance in the neuronal networks responsible for WM in KLS patients, resulting in higher effort at lower WM load, compared with healthy subjects. The general relationship between NAA and BOLD-signal is also discussed in the article.

Introduction

The Kleine Levin Syndrome (KLS) was first described systematically during the early 20th century [1,2]. Presenting mainly during adolescence, it is a rare disorder of periodic hypersomnia associated with behavioral disturbances such as hyperphagia, irritability and hypersexuality. It has a mean duration of eight years and the mean duration of episodes is typically ten days recurring every 3.5 months, with vast inter- and intraindividual variation [3]. At this point, no apparent etiology of the disorder has been detected, and no convincing treatment has been found. Traditionally, patients are considered to have normal function between hypersomnia periods, regarding sleep patterns [4,5] as well as cognitive function. In contrast to this view, we have shown disturbances of complex working memory (WM) that sometimes is long lasting, perhaps even permanent [3,6–8]. Thus, KLS is an in vivo model of the hypothetical association of (periodic) sleep regulation and WM deficit, an association not yet thoroughly examined.

In our KLS patient group, including patients from Nordic countries, single photon emission computed tomography (SPECT)
has shown that the patients often have hypoperfusion in the fronto-temporal areas [6], including areas traditionally linked to WM function [7]. We have also reported that the left lateral prefrontal cortex showed larger Blood Oxygen Level Dependent (BOLD) activation and that the cingulate cortex showed less BOLD-activation in KLS-patients while performing a verbal WM task during functional magnetic resonance imaging (fMRI), compared to healthy control subjects. However, the patients showed increased activity in the left thalamus [9]. In other reports, thalamic hypoperfusion has been observed during episodes, not persisting between hypersomnia episodes, in KLS-patients using SPECT [10]. Other conditions with well-described WM impairment, such as schizophrenia, have shown a similar thalamic activation pattern during WM tasks [11].

Magnetic Resonance Spectroscopy (MRS) is a non-invasive method for investigating brain metabolites in vivo. Some of the most significant metabolites determined using this method are total N-acetyl compounds (tNA) consisting of N-acetylaspartate (NAA) and N-acetylaspartylglutamate (NAAG). These compounds are regarded as markers of neuronal health, viability and number. Total creatine (tCr) consists of creatine and phosphocreatine and reflects energy deposits and metabolism in general. Lipids reflect neuron membrane breakdown. Choline is considered a marker of cellular turnover in malignancy and inflammation. Lactate is a marker for anaerobic metabolism by several underlying pathologies. Myo-inositol (mIns) is a glial marker. Glutamat/glutamine (Glx) is an excitatory neurotransmitter, elevated in several metabolic cerebral conditions [12–14].

Concentrations of the different brain metabolites reflect the pathological status of the tissue and they are modulated in a range of neurological disorders. In idiopathic normal pressure hydrocephalus – a condition associated with WM impairment - there is a marked reduction of NAA in the thalamus [15]. In schizophrenia, also associated with impaired WM, lower NAA concentration compared with healthy controls has been observed [16], although another study has showed no correlation between verbal memory performance and thalamic NAA levels [17]. Regarding MRS investigations in KLS-patients there is, to the best of our knowledge, only a single case report. In this patient, with classical KLS, the authors performed MRS investigations of both hypothalami and thalami inter- and intraictally, i.e. between and during hypersomnic periods. The authors found a difference between the two examinations in the ratios of tNA/Cr and Glx/Cr. They interpreted their findings as indicative of a thalamic dysfunction and that the variation in Glx/Cr-ratio was due to depolarisation block and post-ictal suppression of various thalamic nuclei [18]. A variability in thalamic glutamine between asymptomatic and symptomatic periods has also been shown in a recent case report of a patient also showing varying fMRI patterns to tone stimuli and reduced thalamic perfusion during a symptomatic period [19].

As the thalamus also is involved in sleep regulation [20] the previous findings of thalamic involvement might indicate a neural network linking WM function to sleep regulation. Such linkage is also supported by the fact that higher alertness during a WM task might help in overcoming sleepiness, a finding also positively correlated to higher fMRI-activity in the prefrontal cortices [21].

We previously investigated five individuals with fMRI and MRS during a WM task and found a correlation between the thalamic activation pattern and thalamic NAA-levels (presented at the ISMRM 2009, unpublished data). The major aim with the present study was to clarify if the WM deficit is due to a certain thalamic metabolic disturbance, or if the increased activation is a reflection of a deficit somewhere else in the neural networks of WM. We also aimed at investigating if there is a correlation between a possible disturbance and whether the disease is active or in remission as there are indications that memory disturbances are present even many years after remission. This could be a step in elucidating if the two demonstrated disturbances in KLS, periodic sleep patterns and WM-deficit, are correlated and if so, which one is primary and which one is secondary [6],[9]. The other parts of the brain where our previous study has shown differences in fMRI-activation, prefrontal and cingulate cortices, were not reliably assessable with the MRS-technique of our availability at the time of initiation of this study.

Materials and Methods

Subjects

We examined fourteen patients, six men and eight women, with median/minimum/maximum age of 20.5/14.9/37.3 years. They were diagnosed by a physician with extensive experience of KLS and fulfilled the criteria of KLS according to The International Classification of Sleep disorders, revised by the American Academy of Sleep Medicine 2005. Eight patients had an active disease, defined as at least one symptomatic period during the last one and a half year prior to the examination. The other six patients were regarded as in remission, defined as having no hypersomnia periods for 1.5 years. This interpretation was later confirmed as none of these six patients had any subsequent hypersomnia periods. Patients with active disease were examined during asymptomatic periods, i.e. they had no excessive sleepiness during examinations. No patient was on any medication interfering with CNS-activity for at least a year prior to the examinations. Demographic data are presented in Table 1.

As a control group, we recruited a group of 15 healthy control subjects with a median/minimum/maximum age of 22.1/18.8/40.1 years, all subjects were over 18 years of age for ethical reasons, hence a slight age mismatch.

Working memory function of all subjects was examined in a paper and pencil version of the reading-span task, by Daneman and Carpenter. The results were considered as the baseline WM function of the subjects [22]. Patients and controls were compared using a two-sample t-test.

fMRI and Working Memory

The fMRI-paradigm was presented as previously described [9]. The paradigm consisted of a WM task with four different levels of difficulty. During fMRI-examinations, the subjects were presented with sentences using video-goggles (Resonance Technology Inc, CA, USA). Superlab Pro v 4.0 (Cedrus Corp., San Pedro, USA) was used for presenting the visual WM paradigm. Each sentence remained on the screen for five seconds. The subjects were instructed to press one button if the sentence was correct and another if it was incorrect. One to four different sentences were used in each block. After presentation of the sentences, four words were sequentially shown for 5 s each. The subjects were instructed to indicate if they recognised the word as being the last word of a previously presented sentence, or if it was a new word, not shown before.

All MR measurements were performed using an Achieva 1.5 T MR scanner (Philips, Best, The Netherlands). Functional images were acquired using BOLD-EPI sequence and the standard head coil. The MR-imaging parameters were: echo time (TE) = 40 ms, repetition time (TR) = 2.7 s, flip angle 90°. Thirty-two slices without slice gaps were obtained in interleaved mode. Voxel size was 3 × 3 × 3 mm³ and the number of dynamics (image volumes) was 302.
The ROI center of mass was the left thalamus, which was reported in our previous study [8].

Differences in thalamic activation between KLS patients and healthy subjects were estimated by region of interest (ROI) analysis. The ROI was identified as the significant activation cluster for KLS patients in the left thalamus, which was reported in our previous study [8]. The ROI center of mass was −6; −9; 6 and the volume was 944 mm³. Difference images were obtained by exclusively masking the KLS activation map with the map of healthy controls (mask p-value = 0.05). In the whole brain analysis, an uncorrected threshold of p = 0.001 was used to obtain the difference images using the MarsBaR toolbox [23]. The individual activation levels in the thalamic ROI were calculated as the contrast estimate from non-smoothed images using the MarsBaR toolbox [23]. The individual activation levels during the most difficult condition of the working memory task, which is word recognition after four presented sentences, were calculated.

The BOLD images were preprocessed and analyzed using the SPM5 software (Wellcome Department of Imaging Neuroscience, University College, London, UK). The images were realigned to correct for movement during scanning, normalized and re-sliced to a standard MNI (Montreal Neurological Institute) template. The normalized images were smoothed with a 8 mm Gaussian kernel to ameliorate differences in intersubject localization. Furthermore, the images were analyzed employing a General Linear Model (GLM) and a parametric contrast tapping the different difficulty levels at word recognition after 1–4 presented sentences.

Differences in thalamic activation between KLS patients and healthy subjects were estimated by region of interest (ROI) analysis. The ROI was identified as the significant activation cluster for KLS patients in the left thalamus, which was reported in our previous study [8]. The ROI center of mass was −6; −9; 6 and the volume was 944 mm³. Difference images were obtained by exclusively masking the KLS activation map with the map of healthy controls (mask p-value = 0.05). In the whole brain analysis, an uncorrected threshold of p = 0.001 was used to obtain the difference images using the MarsBaR toolbox [23]. The individual activation levels in the thalamic ROI were calculated as the contrast estimate from non-smoothed images using the MarsBaR toolbox [23]. The individual activation levels during the most difficult condition of the working memory task, which is word recognition after four presented sentences, were calculated.

### Table 1. Clinical characteristics of the included patients.

| No | YoB | Exam date | Gender | Symptoms | Presentation age | Triggers | Ann. freq. | Duration | Active/remission | sWmi | Handedness |
|----|-----|-----------|--------|----------|------------------|----------|------------|----------|-----------------|------|------------|
| 1  | 1988| 2009      | f      | Hs       | 16               | A        | 4–5        | 1–2 w    | r               | Yes  | R          |
| 2  | 1991| 2010      | m      | Hs, Hph, Dp | 13               | none     | 12         | 1½ w    | a               | No   | R          |
| 3  | 1988| 2009      | f      | Hs, Dp   | 15               | none     | 2          | 3–4 w    | a               | No   | R          |
| 4  | 1990| 2010      | f      | Hs, Hph, Dp, Hx, Ha | 14     | none     | 3          | 1–2 w    | r               | No   | R          |
| 5  | 1989| 2009      | m      | Hs, Dp   | 15               | I        | 1–2       | 1 w    | r               | No   | R          |
| 6  | 1993| 2008      | f      | Hs, Dp, Hph | 15     | I, Me     | 2–4       | 1–2 w    | a               | No   | R          |
| 7  | 1979| 2008      | m      | Hs, Hx   | 13               | none     | 7         | 1–2 w    | r               | No   | R          |
| 8  | 1989| 2008      | f      | Hs, Hph  | 16               | A        | 2          | 2 w    | r               | No   | R          |
| 9  | 1972| 2008      | f      | Hs, Hph, Ha | 15     | none     | 6          | 1–2 w    | a               | Yes  | R          |
| 10 | 1994| 2009      | m      | Hs, Dp   | 13               | none     | 2          | 4 w    | a               | Yes  | R          |
| 11 | 1994| 2009      | m      | Hs, Ps   | 14               | I        | 3         | 1 w     | a               | No   | R          |
| 12 | 1988| 2009      | f      | Hs       | 15               | I        | 1–2       | 2–4 w   | a               | Yes  | R          |
| 13 | 1979| 2009      | f      | Hs       | 16               | none     | 1         | 2 w     | a               | Yes  | R          |
| 14 | 1957| 2008      | m      | Hs, Ps   | 16               | none     | 3         | 2 w     | a               | No   | R          |

YoB = year of birth, Ann. Freq = patient appreciated annual frequency of episodes, Duration = duration of episodes, Hs = hypersomnia, Hph = hyperphagia, Ha = hallucinations, Dp = depersonalisation and/or derealisation (as not clearly separated by some patients), Hx = hypersexuality, Ps = psychiatric symptoms, A = alcohol intake, I = infection, Me = Mental exhaustion, sWmi = subjective working memory impairment (asymptomatic periods), R = right.

doi:10.1371/journal.pone.0056279.t001

### Magnetic Resonance Spectroscopy

**Data Acquisition.** The proton MRS data were acquired using the transmit-receive head coil and point-resolved spectroscopy (PRESS) with TE of 25 ms and TR 3 s. 128 water suppressed spectra were averaged, acquisition time 6.54 min:sec, and two MRS volume of interest (VOI) were placed bi-laterally in left and right thalamus (TH) (Fig. 1), the volume o the VOI were adjusted to fit the individual anatomy of each subject with a volume of ca. 3.00 mL. To detect a possible systemic variation of the metabolites we also placed a MRS VOI in the frontal white matter bi-laterally. In the KLS group the min/median/max thalamus volume was 2.02/2.27/3.14 mL. In the control group the min/median/max volume was 2.02/2.27/2.86 mL. T2w coronal images were acquired prior to and after each MRS acquisition in order to determine patient movements. Whole brain coverage quantitative MRI (qMRI) data were acquired using QRAFMASTER sequence with a resolution of 3×1×1 mm³ [24], four echoes and four dynamics using TE = 20.81 ms, TR = 3.7 s. acquisition time 7:05. Min:sec.

**Processing of MR data.** The MRS-data were analyzed using LCModel ver. 6.2-1T [25]. The unsuppressed water signal was used as an internal concentration reference and it was quantified using the water scaling function in LCModel, with the ‘attenuation-of-NMR-visible-water’ (ATTH2O) adjusted to 1.00 (instead of default 0.7). Thus the resulting concentrations were obtained and presented with respect to the aqueous concentrations and completely compensated for by the differences in coil load and temperature, and difference in RF amplification etc. in different subjects. However, the concentrations determined in this manner also depend on the magnitude of the relaxation of the unsuppressed water signal. In order to correct for the relaxation effect, the qMRI-volume were used for this purpose. The qMRI data were processed using SyMRI Brain Studio ver 2 software (SyntheticMR, Linkoping, Sweden). Quantitative maps of R1 ( = 1/T1), R2 ( = 1/T2) and PD covering the brain, were exported as DICOM stacks for further analysis. A multiplicative scaling factor ‘f_{scale}’ was estimated for each MRS (Eq. 1).
Where PD₂,VOI, R₁,VOI and R₂,VOI were the qMRI values within the MRS VOI. Using this scaling factor the estimated concentrations were converted to wet-weight concentrations using the unit mM of aqueous fraction (mM aq.) [26].

**Group Statistic Analysis: MRS.** JMP 8 (SAS Institute Inc, USA) was used to calculate the group statistical analysis. Mean concentrations and standard errors of mean were estimated using a full mixed linear model with the three fixed factors ‘group’, ‘lateral’, ‘anatomical structure’ and all crossing effects. Difference in mean concentrations were then tested using a Student’s t-test and p-values, 0.05 were considered significant.

**Correlation Analysis: MRS and fMRI.** The values of the effect sizes in the left thalamus derived from fMRI were correlated to the estimated metabolite concentration of the left and right thalamus using GraphPad Prism 5.0a (GraphPad Software, San Diego California USA) Pearson correlation, p-values, 0.05 were considered significant.

**Ethics Statement**

The study design was approved by the Regional Ethical Approval Board according to the Swedish Ethical Review Act (2003) in accordance with the Helsinki Declaration of 1975.

**Results**

The main finding of this study was a negative correlation between thalamic activation patterns in fMRI (at the most difficult level in the WM task) and the left thalamic absolute NAA concentration in KLS patients \( r = -0.61, p = 0.022 \) as illustrated in Figure 2. In contrast, no significant correlation between thalamic activation patterns in fMRI and the left thalamic absolute NAA concentration in healthy controls was observed \( r = 0.45, p = 0.14 \). fMRI data from 3 healthy controls were excluded from the correlation analysis due to head movement during fMRI acquisition.

In the pen and paper working memory reading span task, patients recalled less words than the healthy controls; mean 16 (SD = 3.4) vs 22 (SD = 5.7), \( p = 0.01 \). In the fMRI-task, healthy controls scored slightly more correct words than patients \( p = 0.01 \) at level 4 but not at level 1–3 (Table S1).

The fMRI whole brain level analysis confirmed earlier published results of larger activation volume in the left thalamus and the left lateral prefrontal cortex in KLS patients compared to healthy controls during the WM task. However, in the current study, we found the main cortical activation difference in the opercular part of the left inferior frontal gyrus \((-42 4 28, Z = 4.72, cluster size = 442)\) rather than in the triangular part, as reported earlier. KLS patients also had significantly larger activation volume in the bilateral parietal cortex compared to controls: 633 voxels in the left hemisphere with peak at \(-26 -74 52 (Z = 4.02)\) and 631 voxels in the right hemisphere with peak at \(28 -66 30\).
(Z = 4.97). These bilateral parietal clusters encompassed both superior–medial and inferior–lateral aspects of the parietal cortex. Furthermore, in this study we observed that KLS patients had significantly more activation in the bilateral caudate (−16 2 12, Z = 4.26, cluster size = 160; 14 4 12, Z = 4.05, cluster size = 132). The cluster in the left caudate extended into the left thalamus (see below). In addition, the KLS patients had more activation in the bilateral occipital cortex compared to controls. The controls did not have larger activation volume compared to KLS in any cortical areas.

Importantly, the fMRI results confirmed the earlier published differences in thalamic activation between KLS-patients and healthy control subjects (Figures 3 and 4). On a group level, KLS-patients had significantly increased activation in the left thalamus (peak at −8 −4 8, Z = 3.75, cluster size = 31 voxels) during the WM task. As shown in Figure 2, the individual variation of the BOLD signal in the thalamus of KLS-patients is correlated to NAA levels. In contrast, the interindividual variation in the control group was not correlated to NAA levels, or to the WM task result. Thus, no fMRI-activity significantly different from background activity was detected in healthy controls, as was the case in the left thalamus of the KLS-patients [9].

To test the validity of the correlation in the entire KLS group vs. the previously tested group of five individuals (ISMRM 2009), as a control experiment, we repeated the analysis excluding these patients. Exclusion of these patients still resulted in a negative correlation (p = 0.022).

A similar regression analysis for other metabolites showed no significant correlation in any of the groups. In contrast, the correlation was not observed if the analysis was performed using the NAA/Cr-ratio as a relative measure rather than absolute NAA-concentration.

The differences in fMRI activation patterns were not reflected by any significant differences in metabolite concentrations on a group level in either patients or controls as illustrated in Table 2 and Table 3. Neither were there any differences between left and right thalamus within the groups resulting with similar metabolite concentrations for all four areas measured (right and left thalamus in patients and controls respectively). For NAA the mean concentration in the KLS group was 11.33 mM aq. (SE 0.24) and 11.12 mM aq. (SE 0.24) for left and right thalami, respectively, and 11.10 mM aq. (SE 0.25) and 11.15 mM aq. (SE 0.25) for the healthy controls. In frontal white matter, no differences where observed between the groups concerning concentration of any of the metabolites measured.

The distribution of patients in the regression analysis did not show any clustering related to active or remitted disease as illustrated in Figure 2.

Discussion

The finding of a negative correlation between thalamic fMRI-activity and NAA-concentration in the KLS-group and the absence of such a significant variation of fMRI-activity in healthy subjects suggest that there is no simple connection between the concentration of thalamic neurons and the level of thalamic activity during a WM task. The finding can be interpreted as the effect of an effective neurological difference between the KLS patients and healthy controls, for example differences in the dynamics of the networks involved in performing the WM task.

The NAA/Cr-ratios were comparable to the ratios described by Poryazova et al in asymptomatic periods, i.e. 1.3–1.5, albeit they used somewhat different acquisition conditions [18].

A direct comparison between the groups has to take into account the fact that the healthy individuals did not show significantly increased thalamic activity on fMRI. As the patient group showed increased activation upon higher WM task difficulty levels, we hypothesize, based on the findings, that the previously described WM deficit [9] influences the effort needed to complete the task at lower levels. This could imply that with more complex WM tasks, the healthy controls would have a similar activation pattern, possibly resulting in comparable negative correlations to NAA-concentrations. This is supported by the fact that more complex WM tasks have resulted in more complex PET and fMRI activation patterns also in healthy subjects, including increased activity of the thalamus and prefrontal cortices [27–31]. Such an increase can be attributed to a greater need for involvement of the visual attention networks, i.e., the subject has to concentrate harder while performing a more difficult WM task. Neuro-anatomically, “frontal–subcortical circuits” for WM is a well-established concept. These circuits describe projections from the prefrontal cortex to the striatum, globus pallidus, substantia nigra and the thalamus. The thalamus have projections back to the prefrontal cortices forming several loops. These loops have been shown to be involved in both WM and visual attention [32]. The networks of verbal working memory and visual attention both activate the thalamus, but if the two functions are tested more selectively and
with increased WM and visual attention loads, they differ in activation patterns with WM activating more of the prefrontal cortices. This has been interpreted as a need to deploy more executive resources with increasing WM-load and this finding indicates that activation patterns of the networks for WM and visual attention depend on task difficulty [33]. The KLS-patients have not been tested exclusively for visual attention, but in our previous and current studies they show more activation in the left lateral prefrontal cortex, previously shown to be more exclusively attributed to WM [27]. As stated above, the activation patterns in KLS-patients is probably due to an increased effort at lower load, an interpretation that can support a deficit in either of the two principal components of working memory, i.e. working memory and visual attention networks, this since both the thalamus and the prefrontal cortex are increasingly activated in the patient group.

Another important issue to address is the role of NAA in relation to neurons and neuronal function. The somewhat vague terms of “neuronal health, viability and number” was thoroughly reviewed by Moffet et al. in 2007 [13]. NAA is synthesized from aspartate and acetyl-coenzyme A, a metabolism that to this date has been detected in neurons exclusively. In contrast, a theory of NAA having a role in axon-glia signaling has also been proposed; such a role is supported by the fact that catabolic enzymes are located in oligendrodocytes and astrocytes. The theory emphasizes that NAA, which is partly metabolized in glial cells, re-cycle to the neurons, thereby accentuating the neuronal specificity and role of NAA. Even if this inter-cellular metabolism does not show a variability throughout the CNS, there is still a variation of NAA concentration. This is important as it shows that NAA may not be proportionally related to neuronal numbers. As the NAA-absolute quantification method used in this publication estimates NAA based on the water fraction (mM aq.), we prefer the term “neuronal concentration” to the term “neuronal number”. This is supported by the fact that other, non-neuronal specific, metabolites do not covariate with NAA, indicating that the variation is not a result of increased tissue water content, or partial volume effects. The non-specific terms “neuronal health and viability” might nevertheless be partly justified for NAA. A neuronal specific TCA (tricyclic acid) cycle has been proposed, directly linking NAA to the mitochondrial energy metabolism. This, in turn, links NAA to glutamate, which is also dependent on aspartate aminotransferase [13],[34],[35]. As glutamate is proposed to have a significant role in the changing BOLD-signal, stimulating neurons and glial cells to release vascular dilating factors [36,37], aspartate aminotransferase might be linking NAA indirectly to BOLD-signal. Recently, the role of GABA, acting trough inhibitory interneurons or directly on the vasculature, has been a subject of increasing interest, though it has not been shown to be linked with the NAA-concentrations [38].

Table 2. Group mean metabolite concentrations on MRS.

|              | Controls |             | KLS            |             |
|--------------|----------|-------------|----------------|-------------|
|              | TH-L     | TH-R        | TH-L           | TH-R        |
| tCr          | 7.60     | 0.19        | 7.84           | 0.19        |
| NAA          | 11.33    | 0.24        | 11.12          | 0.24        |
| tNA          | 12.54    | 0.26        | 12.38          | 0.26        |
| mIns         | 5.12     | 0.32        | 5.30           | 0.32        |
| tCho         | 2.44     | 0.08        | 2.48           | 0.08        |
| tGlx         | 15.99    | 0.53        | 15.85          | 0.53        |
| NAA/tCr      | 1.50     | 0.05        | 1.43           | 0.05        |
| tNA/tCr      | 1.66     | 0.06        | 1.60           | 0.06        |
| mIns/tCr     | 0.32     | 0.01        | 0.32           | 0.01        |
| tCho/tCr     | 0.67     | 0.05        | 0.68           | 0.05        |
| tGlx/tCr     | 2.12     | 0.11        | 2.06           | 0.11        |

Group mean concentrations and standard error (SE) for metabolites determined by magnetic resonance spectroscopy (MRS). Absolute concentrations are given in mM of aqueous fraction (mM aq.). tCr = total creatine, NAA = n-acetylaspartate, tNA = NAA+ n-acetylaspartateglutamate, mIns = myo-inositol, tCho = total choline, tGlx = glutamate+glutamine. TH-L = left thalamus, TH-R = right thalamus.

doi:10.1371/journal.pone.0056279.t002
With respect to the thalamus, several previous studies have implicated that it is increasingly activated with increased WM demand [29–31]. This has also been shown to be connected to sleep deprivation/state of sleepiness [39]. Other studies have shown that in other disorders with WM deficits, such as normal pressure hydrocephalus, the thalamic absolute NAA-concentrations decrease [15]. To the best of our knowledge, no previous group studies have coupled such findings on fMRI and MRS on an individual level. In other disorders with WM deficits, such as normal pressure hydrocephalus, the thalamic absolute NAA-concentrations decrease [15]. To the best of our knowledge, no previous group studies have coupled such findings on fMRI and MRS on an individual level.

The significant correlation between absolute NAA-concentration and fMRI-activation, in combination with the lack of such correlation coupled such findings on fMRI and MRS on an individual level. To the best of our knowledge, no previous group studies have coupled such findings on fMRI and MRS on an individual level.

The significant correlation between absolute NAA-concentration and fMRI-activation, in combination with the lack of such correlation coupled such findings on fMRI and MRS on an individual level.

Table 3. Differences in group mean metabolite concentrations on MRS.

|                  | KLS TH-L - Controls TH-L | KLS TH-R vs. Controls TH-R | KLS TH-L vs. KLS TH-R | Controls TH-L vs. KLS TH-R |
|------------------|---------------------------|-----------------------------|------------------------|---------------------------|
|                  | Diff SE p                 | Diff SE p                   | Diff SE p              | Diff SE p                 |
| tCr              | 0.088 0.270 1.000         | –0.081 0.270 1.000          | –0.070 0.274 1.000     | –0.240 0.264 0.985        |
| NAA              | –0.227 0.343 0.998        | 0.031 0.343 1.000           | –0.049 0.314 1.000     | –0.209 0.303 0.997        |
| tNA              | 0.013 0.378 1.000         | 0.122 0.378 1.000           | 0.020 0.343 1.000      | 0.161 0.332 1.000         |
| mIns             | –0.291 0.463 0.998        | –0.056 0.463 0.925          | 0.094 0.398 1.000      | –0.178 0.385 1.000        |
| tCho             | –0.147 0.118 0.971        | –0.234 0.118 0.504          | 0.042 0.090 1.000      | –0.045 0.087 1.000        |
| tGlx             | –1.616 0.765 0.413        | 0.167 0.765 1.000           | –1.644 0.771 0.404     | 0.140 0.745 1.000         |
| NAA/tCr          | –0.046 0.075 0.999        | 0.023 0.075 1.000           | –0.001 0.072 1.000     | 0.069 0.070 0.976         |
| tNA/tCr          | –0.024 0.090 1.000        | 0.032 0.090 1.000           | 0.004 0.088 1.000      | 0.059 0.085 0.997         |
| mIns/tCr         | –0.037 0.067 0.999        | –0.075 0.067 0.952          | 0.035 0.056 0.999      | 0.003 0.054 1.000         |
| tCho/tCr         | –0.020 0.020 0.974        | 0.030 0.020 0.811           | 0.012 0.017 0.997      | 0.002 0.016 1.000         |
| tGlx/tCr         | –0.229 0.151 0.798        | 0.024 0.151 1.000           | –0.188 0.151 0.916     | 0.065 0.146 1.000         |

Differences in group mean metabolite concentrations as determined by magnetic resonance spectroscopy (MRS). SE = standard error; tCr = total creatine, NAA = n-acetylaspartate, tNA = NAA + n-acetylaspartate glutamate, mIns = myo-inositol, tCho = total choline, tGlx = glutamate + glutamine. TH-L = left thalamus. TH-R = right thalamus. doi:10.1371/journal.pone.0056279.t003

Table S1 Working memory performance during fMRI. (DOCX)

Supporting Information

Author Contributions

Conceived and designed the experiments: PV AT ME TK OLD PL AML. Performed the experiments: AT ME TK OLD. Analyzed the data: PV AT ME TK OLD PL AML. Contributed reagents/materials/analysis tools: ME PL. Wrote the paper: PV AT ME.
References

1. Kleine W (1925) Periodische Schlafsucht. Monatschrift für Psychiatrie und Neurologie 57: 283–320.
2. Levin M (1936) Periodic somnolence and morbid hunger: A new syndrome. Brain 59: 494–556.
3. Arnulf I, Zeitzer JM, File J, Faber N, Mignot E (2005) Kleine-Levin syndrome: a systematic review of 186 cases in the literature. Brain 128: 2763–2776.
4. Arnulf I, Lin L, Gadotti N, File J, Lecendreux M, et al. (2008) Kleine-Levin syndrome: a systematic study of 108 patients. Am J Neurol 63: 492–493.
5. Huang YS, Guilleminault C, Lin KL, Hwang FM, Liu FY, et al. (2012) Relationship between Kleine-Levin syndrome and upper respiratory infection in Taiwan. Sleep 35: 123–129.
6. Landtblom AM, Dige N, Schwerdt K, Safstrom P, Granerus G (2002) Short-term memory dysfunction in Kleine-Levin syndrome. Acta Neurol Scand 106: 363–367.
7. Wager TD, Smith EE (2003) Neuroimaging studies of working memory: a meta-analysis. Cogn Affect Behav Neurosci 3: 233–274.
8. Landtblom AM, Dige N, Schwerdt K, Safstrom P, Granerus G (2002) A case of Kleine-Levin syndrome examined with SPECT and neuropsychological testing. Acta Neurol Scand 105: 311–321.
9. Engstrom M, Vigen P, Karlsson T, Landtblom AM (2009) Working memory in Kleine-Levin syndrome patients: an fMRI study. Sleep 32: 661–668.
10. Huang YS, Guilleminault C, Kao PF, Liu FY (2005) SPECT findings in the Kleine-Levin syndrome. Sleep 28: 955–960.
11. Bor J, Brunelin J, Suppe-Marmin D, Barrola D, d’Amato T, et al. (2011) Thalamus abnormalities during working memory in schizophrenia. An fMRI study. Schizophr Res 125: 49–53.
12. Ross AJ, Sachdev PS (2004) Magnetic resonance spectroscopy in cognitive research. Brain Res Brain Res Rev 44: 83–102.
13. Moffett JR, Ross B, Arun P, Madhavarao CN, Namhooori AM (2007) N-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. Prog Neurobiol 81: 89–131.
14. Gujar SK, Maheshwari N, Bjorkman-Burtscher I, Sundgren PC (2005) Magnetic resonance spectroscopy. J Neuropsychiatry 23: 217–226.
15. Lundin F, Tsuell A, Dahlqvist Leinhard O, Tullberg M, Wåkkelö C, et al. (2011) Reduced thalamic N-Acetylaspartate in idiopathic normal pressure hydrocephalus: a controlled 1H-magnetic resonance spectroscopy study of frontal deep white matter in the thalamus using absolute quantification. J Neurol Neurosurg Psychiatry 82: 772–778.
16. Endo G, Braus DF, Walter S, Weber-Fahr W, Heun FA (2003) Multiregional 1H-MRS of the hippocampus, thalamus, and basal ganglia in schizophrenia. Eur Arch Psychiatry Clin Neurosci 253: 9–15.
17. Hagino H, Suzuki M, Mori K, Nohara S, Yamashita I, et al. (2002) Proton magnetic resonance spectroscopy of the inferior frontal gyrus and thalamus and its relationship to verbal learning task performance in patients with schizophrenia: a preliminary report. Psychiatry Clin Neurosci 56: 499–507.
18. Parzova R, Schirp B, Roesser P, Basetti CL (2007) Magnetic resonance spectroscopy in a patient with Kleine-Levin syndrome. J Neurol 254: 1445–1446.
19. Billings ME, Watson NF, Keogh BP (2011) Dynamic fMRI changes in Kleine-Levin Syndrome. Sleep Med 12: 533.
20. Murillo-Rodríguez E, Arias-Carrion O, Sanguino-Rodriguez K, Gonzalez-Arias M, Haro R (2009) Mechanisms of sleep-wake cycle modulation. CNS Neurosci Disord Drug Targets 8: 243–253.