doi:10.1093/brain/awu112 Brain 2014: 137; 2077–2087 | 2077

BRAIN
A JOURNAL OF NEUROLOGY

Feeling unreal: a functional imaging study in patients with Kleine-Levin syndrome

Aurelie Kas,[1,2,*] Sophie Lavault,[3,4,5,*] Marie-Odile Habert[1,2] and Isabelle Arnulf[3,4,5]

1 Nuclear Medicine Department, Pitié-Salpêtrière University Hospital, APHP, Paris, France
2 Sorbonne University, UPMC Univ Paris 06, Laboratoire d'Imagerie Fonctionnelle, INSERM UMR S 678, Paris, France
3 Sorbonne University, UPMC Univ Paris 06, Brain Research Institute (CRICM), Inserm UMR-S975, CNRS UMR7225, Paris, France
4 Clinical investigation Centre Paris Est (CIC-9304), Paris, France
5 Sleep Disorders Unit, National Reference Centre for Kleine-Levin Syndrome, Pitié-Salpêtrière University Hospital, APHP, Paris, France

*These authors contributed equally to this work.

Correspondence to: Isabelle Arnulf,
Service des Pathologies du Sommeil,
Hôpital Universitaire Pitié-Salpêtrière,
47-83 boulevard de l'Hôpital,
75651 Paris Cedex 13,
France
E-mail: isabelle.arnulf@psl.aphp.fr

Kleine-Levin syndrome is characterized by relapsing-remitting episodes of severe hypersomnia, cognitive impairment, apathy, derealization and behavioural disturbances. Between episodes, patients have normal sleep, mood and behaviour. Functional imaging studies performed in small series of patients with Kleine-Levin syndrome with visual or semi-quantitative, uncontrolled analysis yielded equivocal brain changes. Using whole brain voxel-based group analysis, we compared brain perfusion scintigraphy during and between episodes in consecutive patients with Kleine-Levin syndrome versus healthy control subjects and correlated perfusion changes with disease severity and symptoms, focusing on less studied but disabling symptoms, such as apathy and derealization. During asymptomatic periods, 41 patients (mean age of 22.3 $\pm$ 8.1 years, 56.1% male) and 15 age- and sex-matched healthy control subjects underwent single-photon emission computed tomography scanning with technetium-99m ethyl cysteinate dimer. Eleven patients repeated the test during a symptomatic period. Compared with controls, patients during asymptomatic periods had persistent hypoperfusion in the hypothalamus, the thalamus (mainly the right posterior part), the caudate nucleus, and cortical associative areas, including the anterior cingulate, (Brodmann area 25), the orbito-frontal (Brodmann area 11) and the right superior temporal cortices (Brodmann area 22), extending to the insula ($P < 0.001$ in all area). Two additional hypoperfused areas emerged during symptomatic periods ($P < 0.001$), located in the right dorsomedial prefrontal cortex (Brodmann area 8) and the right parieto-temporal junction (Brodmann areas 22 and 39). These two areas were more affected between episodes, when the mean episode duration was longer ($r = -0.53$; $P < 0.001$). The score for the Depersonalization/Derealization Inventory during symptomatic periods strongly correlated with the hypoperfusion of the right ($r = -0.74$, $P < 0.001$) and left ($r = -0.59$, $P < 0.005$) parieto-temporal junctions. No hyperperfusion was found. Because the parieto-temporal junction (including the angular gyrus) is involved in cross-modal association between somatosensory (body knowledge), auditory and visual information, the robust hypoperfusions and correlations observed in this area may underlie the striking derealization reported by patients during episodes. Defects in the dorsomedial prefrontal cortex may cause apathy. Persistent hypoperfusion in the diencephalic and associative cortical area during asymptomatic periods is a marker of the disease, suggestive of a scenario wherein patients compensate for these deficient circuitries.

Received December 21, 2013. Revised February 21, 2014. Accepted March 17, 2014. Advance Access publication April 30, 2014

Keywords: derealization; Kleine-Levin Syndrome; apathy; brain scintigraphy
Abbreviation: SPECT = single-photon emission computed tomography

Introduction

With only two cases per million, Kleine-Levin syndrome is a rare neurological disorder that mainly affects adolescents. It is characterized by relapsing-remitting episodes of severe hypersomnia, cognitive impairment, apathy, derealization, and psychiatric and behavioural disturbances, which last usually 1 to 4 weeks (Arnulf *et al.*, 2012). These serious episodes alternate with periods of normal sleep, cognition and behaviour, which typically last several months. The mechanism of Kleine-Levin syndrome is unknown (brain MRI and CSF are unremarkable), but inflammatory or autoimmune hypotheses have been proposed (Carpenter *et al.*, 1982; Dauvilliers *et al.*, 2002). Most meta-analysis initially focused on hypersomnia and visible but less frequent signs such as hypersexuality and megaphagia (reviewed in Arnulf *et al.*, 2005; Billiard *et al.*, 2011); however, a systematic direct interview of 108 patients showed that apathy and derealization affected almost all patients (Arnulf *et al.*, 2008).

Functional imaging studies are key for understanding the basis of these striking neuropsychiatric symptoms; still, they are mostly performed in single cases, difficult to perform during episodes, uncontrolled and sometimes conflicting. Such studies have revealed various hypoperfusions in the thalamic and hypothalamic regions, as well as in the frontal and temporal lobes, some of which persist during asymptomatic periods. In a child with Kleine-Levin syndrome, we previously found a clear hypoperfusion of the left mesiotemporal structures during an episode that persisted one month after the end of the episode using brain perfusion single-photon emission computed tomography (SPECT), with no further difference on subtracted images (Portilla *et al.*, 2002). Two of four patients with Kleine-Levin syndrome had persistent temporal and temporo-frontal cortex hypoperfusions on SPECT during asymptomatic periods, whereas all four had cognitive deficits during asymptomatic periods (Landtblom *et al.*, 2003). In another SPECT study performed during asymptomatic periods in seven children with Kleine-Levin syndrome, the individual semi-quantitative analysis identified some local persistent hypoperfusions in only two of seven patients, affecting the left anterior temporal cortex in one patient and the temporal lobe, the right posterior frontal lobe, bilateral parietal and occipital lobes, and the left basal ganglia in the other patient (Huang *et al.*, 2005). A single patient had a hypoperfusion in the right mesial temporal region during an asymptomatic period (Hong *et al.*, 2006). One may note that the most consistent finding in the three studies using visual or semiquantitative analysis was the reduced temporal lobe perfusion during asymptomatic period. During symptomatic episodes, left/right visual SPECT comparisons of the basal ganglia in 27 individual children with Kleine-Levin syndrome showed that 67% of the cases had asymmetric hypoperfusion in the left thalamus, 11% in the right thalamus, 11% in the left basal ganglia and 22% in the right basal ganglia (Huang *et al.*, 2012). However, other subcortical or cortical areas were not mentioned, and no subtractions were performed during symptomatic versus asymptomatic periods. During the symptomatic episodes, two patients with Kleine-Levin syndrome exhibited a decreased metabolism in the hypothalamus, the orbitofrontal and frontal parasagittal areas, and the bilateral posterior regions, as well as increased caudate, cingulate, and premotor metabolism detected by ^{18}F-fluorodeoxyglucose-PET compared with asymptomatic periods, although no change was observed in the thalami (Haba-Rubio *et al.*, 2012).

Overall, the previous SPECT or PET studies in Kleine-Levin syndrome are hampered by the small number of subjects or by the absence of quantitative image assessment and a control group to conduct between groups' comparisons. They also highlight a large variability of Kleine-Levin syndrome clinical and imaging presentations. Our first purpose was to explore the brain perfusion patterns and disease related-changes in a large group of patients with Kleine-Levin syndrome, using an automated voxel-based method with statistical parametric mapping. This approach is able to enhance common brain alterations in a large group of subjects and has several advantages over visual- or region of interest-based assessments, because the analysis is performed across the whole brain with minimal user bias. In addition, the method of clinical/imaging correlations allows the study of the anatomical correlates of a cognitive process assessed by a specific test in patients with neurological disorders (Desgranges *et al.*, 2002). Still, this approach has not yet been used in patients with Kleine-Levin syndrome. We compared brain perfusion scintigraphy during and between episodes in consecutive patients with Kleine-Levin syndrome versus healthy control subjects, analysed the brain perfusion profiles according to the disease severity and explored the neural bases of symptoms by studying the voxel-based correlations between SPECT and symptoms scores, focusing on less-studied but disabling symptoms, such as apathy and derealization.

Materials and methods

Subjects

One hundred and five patients were referred to the national reference centre for suspicion of Kleine-Levin syndrome between 2007 and 2011 (Figure 1). Seventy patients met the international criteria of primary Kleine-Levin syndrome (American Academy of Sleep Medicine, 2005) and were regularly followed in the centre. Among them, 41 patients underwent a brain perfusion SPECT with technetium-99m ethyl cysteinate dimer (99mTc-ECD) between episodes as part of their routine clinical evaluation. The other patients had functional brain imaging with 99mTc-exametazine ($n = 3$, not analysed here) and no imaging ($n = 26$).

These 29 patients did not differ in age, sex, or severity of the disease (number of episode and cumulated duration of episode) from the sample of 41 patients studied here. In addition, 13 of 41

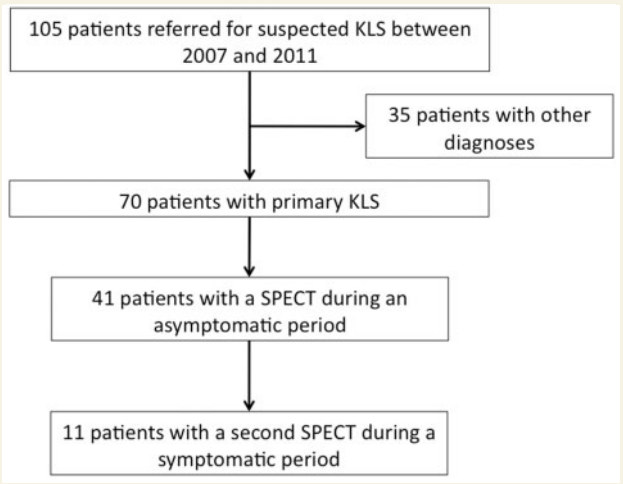

Figure 1 Flow chart of patients with Kleine-Levin Syndrome (KLS).

patients were able and agreed to come to the centre during an episode for a second brain imaging, as the main limitation for this exam was the distance between their home and the centre. In two of these patients, the image acquisitions were impossible to perform because the patients were agitated and delusional. Symptomatic brain SPECT imaging was performed 1 to 22 days after the onset of an episode and was separated from asymptomatic SPECT by 40 days to 8 months. Fifteen young, unrelated healthy control subjects (mean age 22.6 ± 4.7 years, range 18.7–35.2 years; nine males and six females) were recruited by the Clinical Investigation Centre Paris-Est (through their list of healthy control subjects obtained by advertisement), and compensated for taking part in the SPECT study. We could not recruit more controls because of the sudden worldwide cessation of 99mTc-ECD production. All participants (plus the parents of minors) signed a written consent for the study. The research program was approved by the ethics committee (Comité de Protection des Personnes Ile de France 06).

Clinical measures

The patients were examined by I.A., and completed the Stanford Kleine-Levin syndrome questionnaire (Arnulf *et al.*, 2008), the Epworth sleepiness score (Johns, 1994) and the Hospital Anxiety and Depression Rating Scale (Zigmond and Snaith, 1983), the Depersonalization/Derealization Inventory (Cox and Swinson, 2002) and the apathy scale of Starkstein, in and out of episodes (Starkstein *et al.*, 1992). Later, we asked a consecutive series of 30 patients to specify the nature of abnormal perceptions during an episode by administrating a systematic inventory questioning vision (disturbed, loss of 3D vision, colour, contrast, perception of movement), audition (disturbed, frequency change, sound intensity), olfaction (disturbed), tactile sensations (disturbed, impaired sense of friction), and pain (heat/cold discrimination). Symptoms were reported during at least one episode in the questionnaire and at the time of the SPECT during the symptomatic period. The analyses of morphological brain magnetic resonance imaging were unremarkable, except for incidental findings in one patient with a mild ventricular dilatation and a mild atrophy of the corpus callosum in the splenium area. At time of the symptomatic SPECT, patients were treated with lithium ($n = 4$), valproic acid ($n = 2$, combined with amisulpiride in one patient), oxcarbazepin ($n = 1$), or fluoxetine ($n = 1$); three patients did not have any treatment. The treatments were kept at a stable dosage in the symptomatic and asymptomatic periods.

Brain perfusion single-photon emission computed tomography

Image acquisition, reconstruction and post-processing were identical during the asymptomatic and symptomatic periods. One hundred and twenty projections were acquired 30 min after the intravenous injection of 99mTc-ECD (740 MBq) in a 128 × 128 matrix with a three-headed gamma camera equipped with parallel, high resolution collimators (Irix, Philips Medical Systems). The projections were reconstructed using an iterative algorithm, post-filtered and corrected for attenuation using the Chang method (attenuation coefficient $\mu = 0.12\,cm^{-1}$). The reconstructed volumes were spatially normalized to the Montreal Neurological Institute space with Statistical Parametric Mapping software (SPM8, Welcome Department of Cognitive Neurology, University College, London), using a SPECT perfusion template. A 12-parameter affine transformation was used, before a non-linear estimation of the deformations required for an optimal registration. Normalized images were smoothed using an isotropic Gaussian kernel of 12 mm. The dimensions of the resulting voxel were $2 \times 2 \times 2\,mm^3$. Global normalization was performed using proportional scaling to a global value of cerebral blood of 50 ml/min per 100 *g*.

Statistical analysis

The clinical measures were compared between groups using chi-square tests for qualitative measures and Student *t*-tests for quantitative measures. The clinical differences during the symptomatic versus asymptomatic periods within the Kleine-Levin syndrome group were assessed using paired *t*-tests. Correlations between clinical scores were performed using the Pearson correlation test.

The brain perfusion profiles were compared between patients with Kleine-Levin syndrome in asymptomatic state and healthy volunteers, using a two-sample *t*-test on the voxel level with SPM8 software. The statistical significance threshold was set at $P < 0.001$ uncorrected for multiple tests. Only clusters of more than 100 voxels were considered. The specific perfusion changes in symptomatic versus asymptomatic periods were determined in the subgroup of 11 patients using a paired *t*-test. The voxel-based brain perfusion was correlated with the disease course (measured in years), the mean duration of symptomatic episodes (in days), and the apathy and derealization scores during asymptomatic periods, independently of age. Considering the small sample size of patients, SPM T-maps were displayed at a height threshold of uncorrected $P < 0.005$, which is an accepted procedure in correlation analyses considering the number of subjects (Desgranges *et al.*, 1998). Then, individual adjusted normalized regional activities values were extracted from the eligible clusters to calculate correlation coefficients using the MarsBaR software (http://marsbar.sourceforge.net/).

Results

Symptoms

The demography and clinical characteristics of patients and controls at time of the study are shown in Table 1. There were

Table 1 Demographic and clinical characteristics of patients tested with SPECT during asymptomatic and symptomatic periods, and controls

	Patients with SPECT during asymptomatic period	Patients with SPECT during asymptomatic and symptomatic periods	Healthy controls
n	41	11	15
Age at time of study, years	22.3 ± 8.1 (11.4–51.8)	23.0 ± 10.8 (14.4–51.8)	22.6 ± 4.7 (18.7–35.2)
Sex, % male	56.1	54.5	60.0
Right-handed, %	85	72.7	100
Prematurity (<8 months), %	29.3	27.3	0.7
Birth problems[a], %	31.7	54.5	0*
Developmental delay, %	9.8	18.2	0
Education level, 1–7	4.9 ± 1.5	4.3 ± 1.3	6.0 ± 0.9
Sleepiness score, 0–24	6.2 ± 4.8	7.8 ± 5.6	7.0 ± 2.6
Anxiety and depression score, 0–14	9.2 ± 6.1	10.1 ± 7.6	7.5 ± 3.9
Age at disease onset, years	16.4 ± 5.4 (9.5–44.6)	17.9 ± 9.4 (9.5–44.6)	NA
Disease course, years	5.9 ± 5.8 (0.2–25.6)	5.0 ± 4.2 (0.3–10.8)	NA
Time since last episode, days	51 ± 59	56 ± 70	NA
Number of episodes	14.0 ± 12.8 (2–50)	14.5 ± 12.2 (4–41)	NA
Time incapacitated, days	197 ± 220 (20–800)	249 ± 241(70–651)	NA

NA = not applicable. Data are mean ± SD (range), otherwise specified; *$P < 0.05$ for a difference between controls and patients. There were no differences between the two patient subgroups.
[a]Prematurity ($n = 12$), difficult labour requiring Cesaerian section ($n = 5$), lack of oxygen at birth or umbilical-cord around the neck ($n = 4$), use of forceps ($n = 3$).

no differences between the 30 patients who had only an asymptomatic SPECT and the 11 patients studied both during asymptomatic and a symptomatic periods. Some triggering factors at the onset of Kleine-Levin syndrome were reported in 37 (90.2%) patients, including infection (upper airways infection, $n = 10$, gastroenteritis, $n = 5$, fever, $n = 2$) in 41.5% patients, jet lag or sleep deprivation in 17 (41.5%) patients, alcohol intake in 10 (24.4%), stressful events in four (9.8%), travel without any sleep perturbation in four (9.8%), head injury in three (7.3%), general anaesthesia in two (4.9%), and trip at high altitude in one (2.4%). The same factors plus menstruation ($n = 4$), marijuana intake ($n = 2$), and staphylococcal pelvic infection ($n = 1$) triggered some recurrent episodes. The specific episode with a symptomatic SPECT was triggered by an infection in two patients (amygdalitis, $n = 1$, bronchitis, $n = 1$), an acute sleep deprivation in one patient and nothing identifiable in eight patients.

The symptoms occurring during at least one episode in the 41 patients are shown in Fig. 2.

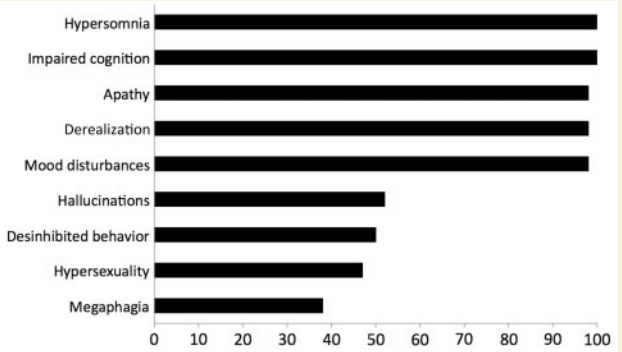

Figure 2 Symptoms having occurred during at least one episode in the 41 patients with Kleine-Levin syndrome.

The apathy scores were higher during symptomatic than asymptomatic periods (30.4 ± 8.3 versus 9.5 ± 4.6; $P < 0.0001$). During symptomatic periods, patients did not seek novelty and stopped their usually pleasant activities, including reading, watching TV, playing videogames, seeing friends or using social networks, using their cell phone and sending short messages. They exhibited a withdrawn attitude (79%; $n = 31/39$), neglected their hygiene (79%; $n = 31/39$) and lacked motivation (72%; $n = 28/39$). As for derealization, almost all patients (97.6%; $n = 40/41$) felt that their perceptions were unreal or changed, as if in a dream (85%; $n = 33/39$), with an unreal feeling of not being themselves (82%; $n = 32/39$) and of being partly detached from the environment (74%; $n = 29/39$) or from their body (54%; $n = 21/39$) but lacking any heautoscopy (seeing one's proper body from an external perspective). A patient described himself as living in a parallel world without being an actor in his life. Another had the feeling of not being alive. As the sun was shining, another patient asked his mother: 'Is it day or night'? Patients had a higher score in versus out of the episodes in the Depersonalization/Derealization Inventory (70 ± 22.2 versus 13.1 ± 17.9; $P < 0.0001$, Supplementary Table 1). There was no significant correlation between the derealization score and the apathy score, both during symptomatic and asymptomatic periods. Among the 30 patients with a specific interview on perceptions, most (70%) reported abnormal visual perceptions during an episode, along with a difficulty evaluating and detecting the movement of cars or other

objects (73%), seeing space in three dimensions (43%), and discerning contours (30%), colours (17%) or contrasts (13%). They had unusual food tastes (63%, 19/30), abnormal smells (50%), and disturbed hearing (73%) for sound intensity (73%) and frequency (10%). Moreover, 70% of patients had abnormal tactile perceptions with an increase in pain threshold (58%) or friction less acutely on their skin (57%). They had a difficulty distinguishing cold from hot water (63%). This also included some striking reports of difficulties dealing with cross-modal sensory stimulations. Some patients saw an interlocutor speaking without hearing his words at the same time as his lips were moving (like a badly dubbed movie). Several patients reported that the showering experience was disagreeable because they could see water flowing on their body without feeling it exactly at the same time, in addition to major difficulties evaluating its temperature. They did not like looking at their faces in the mirror, as they had difficulties recognizing themselves.

At the time of symptomatic SPECT in 11 patients, the symptoms included hypersomnia [$n = 11$ (100%), with a subjective mean 16 ± 4.3 h of sleep/day], major asthenia ($n = 11$), derealization ($n = 11$), apathy ($n = 11$), attention difficulties ($n = 10$), slowed speech ($n = 10$), anxiety ($n = 9$), difficulty reading ($n = 9$) and performing two tasks simultaneously ($n = 8$), depressed mood ($n = 8$), irritability ($n = 7$), difficulty making decisions ($n = 7$), hallucinations ($n = 6$), clumsiness ($n = 6$), anorexia ($n = 5$), baby-like speech ($n = 5$), intense dreaming activity ($n = 4$), hypersexuality ($n = 4$), behavioural agitation ($n = 3$), hyperphagia ($n = 3$) and regressive behaviours requiring their mother's attention ($n = 2$). Notably, there was no hemispatial neglect, hemiasomatognosia, acalculia, agraphia or left-right disorientation. These 11 patients studied in asymptomatic versus symptomatic periods had similar demography and clinical characteristics as the whole Kleine-Levin syndrome group (Table 1).

Single-photon emission computed tomography analysis during asymptomatic periods

The asymptomatic SPECT was performed after a mean 51 (1–277) days after the end of an episode in the 41 patients. Compared with age-matched controls, there was a hypoperfusion in the bilateral thalami (especially the right posterior part), the hypothalamus, and bilateral basal ganglia (especially the left caudate nucleus) (all $P < 0.001$). Cortical hypoperfusion was observed in the orbitofrontal cortex [Brodmann area (BA) 11], the anterior cingulate (BA25), and the left superior temporal gyrus (BA22), extending to the insula (all $P < 0.001$, Table 2 and Fig. 3). Notably, the thalamus was also hypoperfused during the asymptomatic period in the subgroup of 11 patients compared to controls ($P < 0.001$, data not shown). No hyperperfusion was found. To determine whether the recent termination of an episode would influence the SPECT results, we restricted the analysis to the 36 patients who had finished their last episode for at least 5 days and found the same results (data not shown). Results were also similar when restricted to the 36 right-handed patients.

Table 2 Between-group analyses of brain perfusion SPECT: results of the statistical parametric mapping

Location (BA)	Coordinates *x*, *y*, *z* (mm)	Z score (K_E)	$P_{uncorrected}$
Hypoperfusion in the Kleine-Levin syndrome group in asymptomatic period ($n = 41$) versus the age-matched control group ($n = 15$)			
Right thalamus	**4, −25, −2**	**4.7 (343)**	**<0.001**
Anterior cingulate (BA25)	**2, 11, −4**	**4.8 (1307)**	**<0.001**
Hypothalamus	−6, −3, −12	4.0	<0.001
Left caudate nucleus	−8, 16, 5	3.7	<0.001
Left superior temporal gyrus (BA22)	**−51, 6, −4**	**4.2 (517)**	**<0.001**
Rectus gyrus (BA11)	**0, 57, −18**	**3.5 (272)**	**<0.001**
Rectus gyrus (BA11)	0, 32, −20	3.7	<0.001
Left orbital gyrus (BA11)	−2, 42, −22	3.4	<0.001
Hypoperfusion in the Kleine-Levin syndrome group in the symptomatic period ($n = 11$) versus the age-matched control group ($n = 15$)			
Right thalamus	**6, −25, −1**	**4.1 (129)**	**<0.001**
Right cuneus (BA18/31)	**8, −71, 20**	**4.1 (318)**	**<0.001**
Left inferior frontal gyrus (BA47)	**−32, 19, −18**	**3.9 (187)**	**<0.001**
Left superior temporal gyrus (BA38)	−44, 22, −20	3.6	<0.001
Right medial frontal gyrus (BA11)	**2, 61, −13**	**3.5 (114)**	**<0.001**
Hypoperfusion in the Kleine-Levin syndrome group in the symptomatic period compared with the asymptomatic period ($n = 11$)			
Right medial frontal gyrus (BA8)	**2, 34, 50**	**7.9 (109)**	**<0.001**
Right superior temporal gyrus (BA22)	**46, −50, 15**	**4.3 (132)**	**<0.001**
Right angular gyrus (BA39)	44, −51, 23	3.6	<0.001

Coordinates are in millimetres relative to the anterior commissure, corresponding to the atlas of Talairach and Tournoux. Statistical maps were thresholded for significance at $P < 0.001$ uncorrected for multiple tests, with a cluster extent of 100 voxels. K_E = number of voxels per significant cluster. Data related to the cluster local maxima are in bold. BA = Brodmann area.

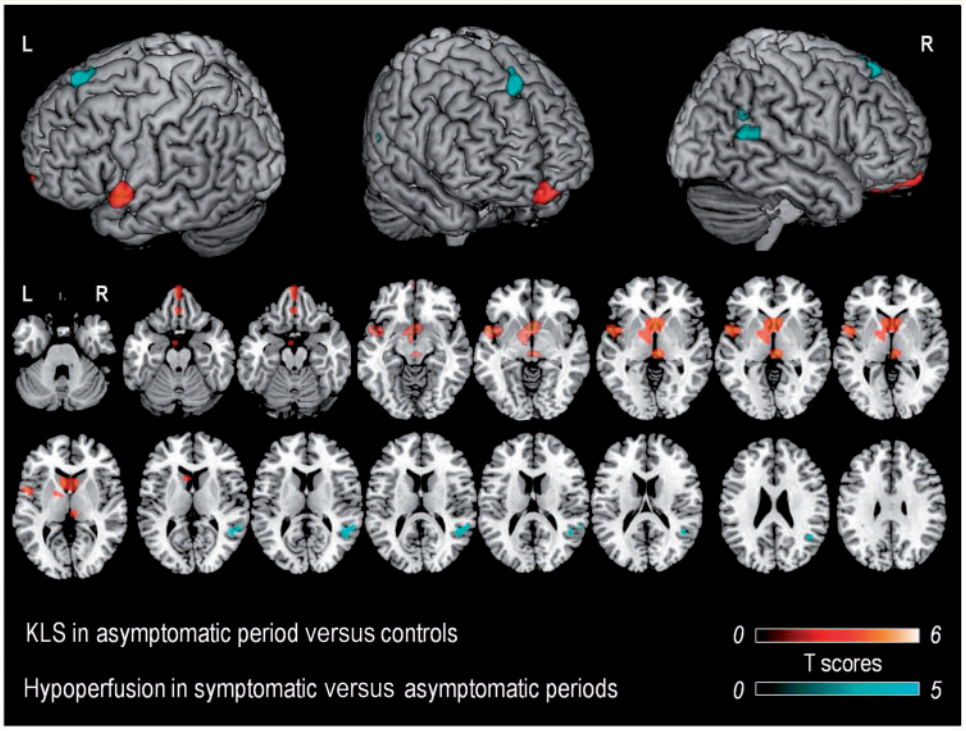

Figure 3 Brain hypoperfusion patterns in asymptomatic period [$n = 41$ patients with Kleine-Levin syndrome (KLS)] versus controls ($n = 15$) (hot colours): the perfusion is significantly decreased in the orbitofrontal cortex, the left superior temporal region and in the insula. Subcortical perfusion is decreased in the thalamus, the hypothalamus, and in the caudate nucleus ($P < 0.001$ uncorrected). Regional hypoperfusion occurring in symptomatic versus asymptomatic periods in 11 patients with Kleine-Levin syndrome (cold colours): during episode, a hypoperfusion is observed in the dorsomedial prefrontal cortex (BA8) and the right parieto-temporal junction (BA22 and BA39). The SPM T-maps are projected onto a surface rendering and onto axial views of the customized MRI template. The axial slices are shown using neurological conventions (right is right). R = right; L = left; BA = Brodmann area.

Single-photon emission computed tomography changes during symptomatic periods

Compared with control subjects, the symptomatic SPECT showed a hypoperfusion in the right thalamus (mostly in its posterior part), in the associative occipital cortex (BA18/31 junction), and in the orbitofrontal cortex (BA11 and 47) (all $P < 0.001$, Table 2). The symptomatic versus asymptomatic SPECT comparison revealed two additionally observed hypoperfused areas emerging during symptomatic periods located in the dorsomedial prefrontal cortex (BA8) and in the posterior part of the right superior temporal (BA22), extending to the middle temporal (BA21) and the angular gyrus (BA39) (all $P < 0.001$, Table 2 and Fig. 3). Other hypoperfused areas were found at a threshold of $P < 0.005$ (uncorrected) in the anterior cingulate (BA32) extending to the orbitofrontal cortex (BA11). There was no hyperperfused area during symptomatic periods, whether versus asymptomatic periods of versus controls. To test the robustness of the results, we restricted the comparisons to the 10 patients who had an asymptomatic scanning after a minimum 6 days since last episode and found the same results (data not shown). We also excluded from the analysis the single patient who was older than 20 at disease onset and found the same results (data not shown).

Correlations between clinical measures and brain perfusion during asymptomatic periods

The hypoperfusion in the left middle orbital frontal cortex (BA10/11), but no other area, improved with time since last episode (Table 3 and Fig. 4). The perfusion during the asymptomatic period in the right parieto-temporal (BA39 and 40, correlation coefficient $r = -0.53$, $P < 0.001$) and dorsomedial prefrontal cortices (BA8, $r = -0.54$, $P < 0.001$) decreased with increasing duration of episodes (but not the number of episodes) (Table 3 and Fig. 5). In addition, there was a tendency toward decreased perfusion in the right parieto-temporal cortex with longer disease course (in years) ($P < 0.06$, Table 3).

The Depersonalization/Derealization Inventory score during symptomatic periods strongly correlated with the perfusion of the bilateral associative posterior cortex (Figu. 6), especially the posterior part of the middle temporal gyrus, showing a left predominance (BA39, $r = -0.74$, $P < 0.001$). The Depersonalization/Derealization Inventory score measured during asymptomatic periods correlated with the perfusion in the left temporo-occipital cortex (BA39, $r = -0.45$, $P < 0.05$). No correlation was observed between brain perfusion and apathy scores (Table 3).

Table 3 Voxel-based correlations between brain perfusion and clinical measures in Kleine-Levin syndrome

Location (BA)	Coordinates *x*, *y*, *z* (mm)	Z-score (K_E)	Correlation coefficient (*P*-value)
Correlation between brain perfusion and the time since last episode in days ($n = 41$)			
Left middle orbital frontal gyrus (BA10/11)	−8, 66, −7	3.7 (104)	0.48 (0.002)
Correlation between brain perfusion and the mean duration of the episodes ($n = 41$)			
Right middle temporal gyrus (BA39)	50, −57, 23	3.7 (313)	−0.53 (<0.001)
Left precentral gyrus (BA6)	−42, −2, 35	3.7 (145)	−0.52 (<0.001)
Left medial frontal gyrus (BA8)	−4, 39, 50	3.6 (252)	−0.54 (<0.001)
Right inferior parietal (BA40)	30, −45, 39	3.3 (279)	−0.50 (<0.001)
Correlation between brain perfusion and the mean duration of the disease ($n = 41$)			
Right precentral gyrus (BA6)	−63, −12, 34	4.0 (179)	−0.32 (<0.05)
Right middle temporal gyrus (BA21)	65, −41, −1	3.7 (465)	−0.15 (0.06)
Correlation between brain perfusion and the derealization/depersonalization score during symptomatic periods ($n = 23$)			
Left middle temporal gyrus (BA39)	−46, −66, 11	3.8 (520)	−0.74 (<0.001)
Right middle temporal gyrus (BA39)	38, −55, 25	3.4 (111)	−0.59 (<0.005)
Left inferior occipital gyrus (BA18)	−26, −91, −1	3.3 (164)	−0.54 (<0.01)
Right precuneus (BA7)	4, −53, 65	3.0 (132)	−0.58 (<0.005)
Correlation between brain perfusion and the derealization/depersonalization score during asymptomatic periods ($n = 25$)			
Left middle temporal gyrus (BA39)	−30, −65, 14	4.8 (367)	−0.45 (<0.05)

Coordinates are in millimetres relative to the anterior commissure, corresponding to the atlas of Talairach and Tournoux. SPM T-maps were thresholded for significance at $P < 0.005$ uncorrected for multiple tests, with a cluster extent of 100 voxels. K_E = number of voxels per significant cluster. BA = Brodmann area.

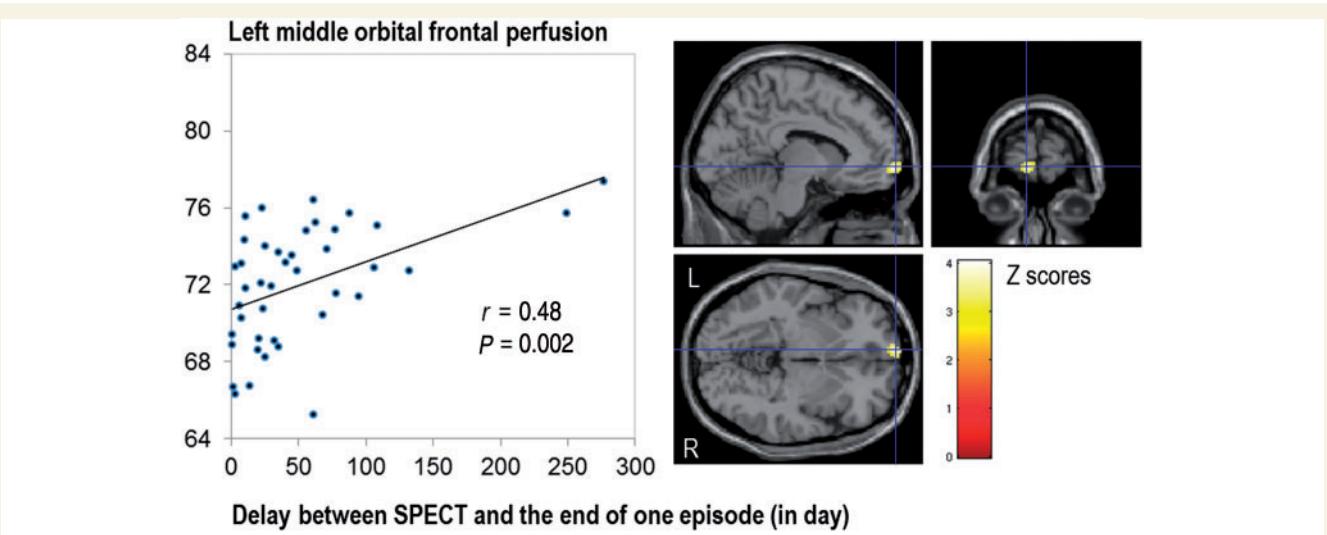

Figure 4 Correlation between the left orbital frontal perfusion and the time since last episode. Plots of the normalized perfusion values in the left orbital frontal gyrus (BA10/11) in patients with Kleine-Levin syndrome for the cluster ($x = -8$, $y = 66$, $z = -7$ in the Talairach Atlas) obtained from correlation with the time since the end of the last episode. The normalized perfusion values are expressed as a percentage of global cerebral blood flow. SPM T-maps were generated at $P < 0.002$ uncorrected, with cluster extend of 100 voxels. R = right; L = left.

The voxel-based correlations between perfusion in one hand, and the duration of episodes in days, the derealization score, and the hypoperfused areas occurring with symptoms in the other hand point out the involvement of the posterior associative cortex, especially the posterior part of the right middle temporal cortex, in BA39 (Fig. 7). Notably, we found no correlation between the time asleep during episodes and the SPECT hypoperfusion, whether during symptomatic and asymptomatic periods, nor with the residual Epworth sleepiness score during asymptomatic periods. The anxiety and depression score did not correlate with any brain hypoperfusion.

Discussion

Patients with Kleine-Levin syndrome demonstrated a persistent hypoperfusion within the thalamus, the hypothalamus and the caudate nucleus, as well as within associative cortical areas,

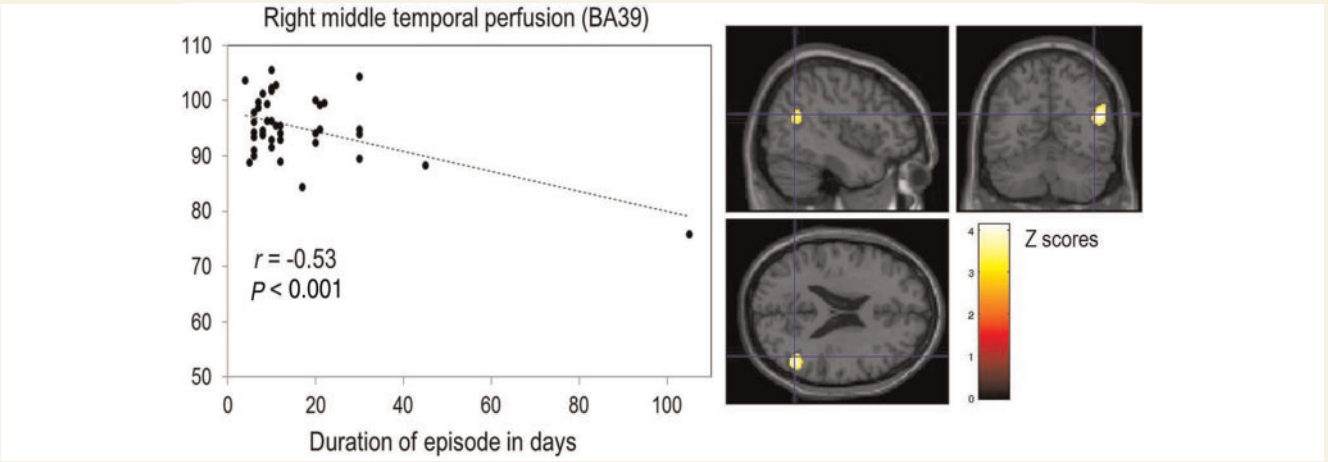

Figure 5 Correlation between the right middle temporal perfusion and the duration of episodes. Plots of the normalized perfusion values in the right middle temporal region (BA39) in patients with Kleine-Levin syndrome for the cluster ($x = 50$, $y = -57$, $z = 23$ in the Talairach Atlas) obtained from correlation with the duration of episode in days. The normalized perfusion values are expressed as a percentage of global cerebral blood flow. SPM T-maps were generated at $P < 0.005$ uncorrected, with cluster extend of 100 voxels.

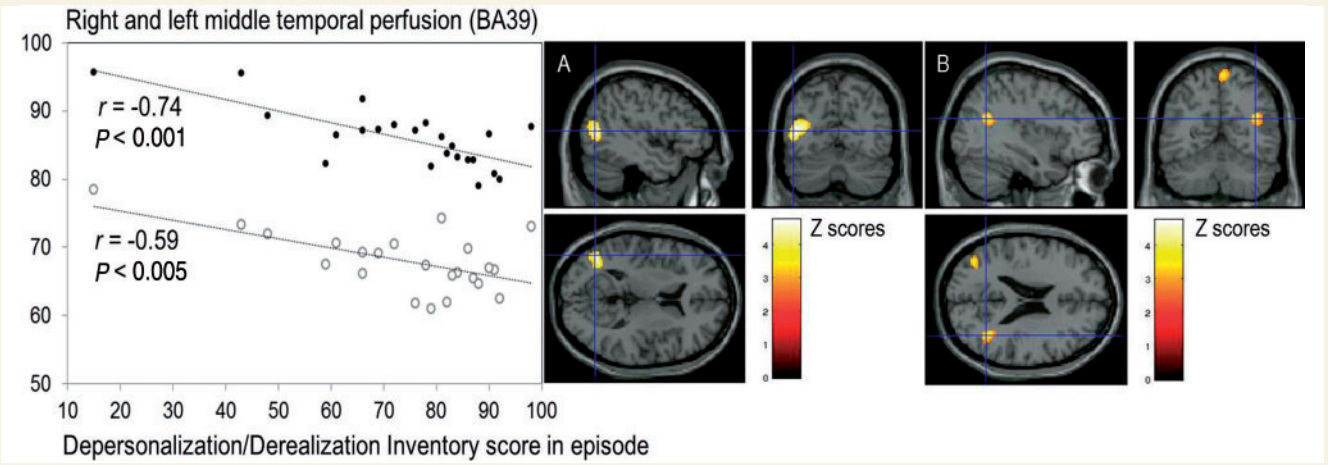

Figure 6 Correlation between the middle temporal perfusion and the derealization depersonalization inventory scores during symptomatic periods. Plots of the normalized brain perfusion values in the left middle temporal regions ($x = -46$, $y = -66$, $z = 11$, filled circles and **A**) and in the right ($x = 38$, $y = -55$, $z = 25$, open circles and **B**) obtained from voxel-based correlation with Derealization/Depersonalization Inventory scores during symptomatic periods in patients with Kleine-Levin syndrome. SPM T-maps were generated at $P < 0.005$ uncorrected, with cluster extend of 100 voxels.

including the orbito-frontal, insular, anterior cingulate and left temporal cortices, during asymptomatic periods compared with controls. Two additional hypoperfused areas emerged during the symptomatic period in the dorsomedial prefrontal cortex and in the right parieto-temporal junction (including the posterior temporal cortex and the angular gyrus). Increased duration of episodes and longer disease course predicted a decreased perfusion at the right parieto-temporal cortex junction, whereas the increased duration of episodes also predicted a dorsomedial prefrontal hypoperfusion during asymptomatic periods. The orbital-frontal hypoperfusion improved with time since last episode. No region was hyperperfused, either in symptomatic versus asymptomatic periods, in symptomatic periods versus controls or in asymptomatic patients versus controls.

Abnormalities during asymptomatic periods

The main result is the persistent hypoperfusion in several subcortical and cortical areas, observed in a large group of asymptomatic patients compared with young healthy volunteers. Until this voxel-based group study, brain perfusion scintigraphy was analysed in smaller samples by visual inspection or semi-quantitative approaches. The involvement of three major subcortical structures during asymptomatic periods (the hypothalamus, the posterior thalamus and the caudate nucleus, as well as the associative cortex, including the orbitofrontal cortex and the left superior temporal gyrus (extending to the insula) is a new finding and raises the question of their clinical impact.

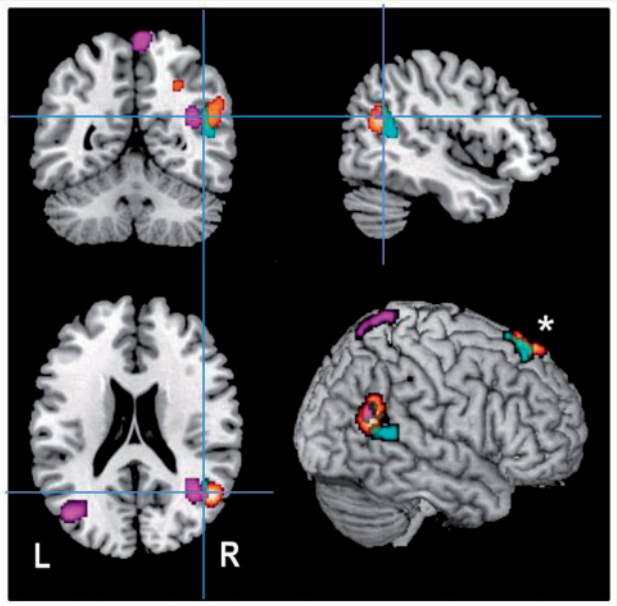

Figure 7 Right middle temporal dysfunction related to symptomatic period in Kleine-Levin syndrome. The figure shows the voxel-based correlations between perfusion, and the duration of episodes in days (in yellow to red tints), the derealization score (in violet tints), and the hypoperfused areas occurring with symptoms (in cyan tints). These results point out the involvement of the posterior associative cortex, especially the posterior part of the right middle temporal cortex (BA39) in the Kleine-Levin syndrome symptoms, the derealization feeling and their duration. Perfusion changes in dorsomedial prefrontal cortex (shown by the asterisk) are also involved in episode and are strongly associated with its duration. R = right; L = left.

Notably, the abnormal functional imaging contrasted with almost normal reported sleep, mood and cognition during asymptomatic periods in our patients. There may, however, exist some subtle residual alteration of cognitive abilities in patients with Kleine-Levin syndrome with or without complaints that would be detected only by complex formal tests (Landtblom *et al.*, 2003). In addition, patients with Kleine-Levin syndrome performing a working memory task needed to recruit a different network (including increased thalamic activity and decreased cingular activity and adjacent prefrontal cortex, as shown by functional MRI) to achieve similar or lower performances than controls, suggesting a more effortful process in patients (Engström *et al.*, 2009, 2013; Vigren *et al.*, 2013). We suggest that the hypoperfusions observed in Kleine-Levin syndrome did not translate into complaints during asymptomatic periods because most patients compensated for these deficits, depending on their personal cognitive and psychological reserves. They should, however, be studied in greater detail for any effects in the long term psychological and cognitive domains, because these brain dysfunctions may have long-term impacts.

Two pieces of evidence here suggest a cumulative impact of episodes on brain functioning. The right parieto-temporal junction and the dorsomedial prefrontal cortex were more affected between episodes, when the disease course and mean episode duration were longer, and the same areas were more affected in symptomatic than in asymptomatic periods. Interestingly, the more the patients suffered from derealization during episodes, the more the bilateral associative posterior cortex (especially the right parieto-temporal cortex) remained affected after the episode. Therefore, this brain perfusion defect may be a residual marker of the severity of episodes. All in all, these results suggest that Kleine-Levin syndrome is not an episodic, but rather a continuous and durable condition that is exacerbated during symptomatic periods. The improvement of orbitofrontal hypoperfusion with longer time since last episode, however, suggests that the brain partially recovers with time.

Abnormalities during symptomatic periods

During symptomatic periods, two additional brain areas are affected, including the dorsomedial prefrontal cortex (BA8) and the posterior part of the right temporal cortex (BA21 and 22), extending to the angular gyrus (BA39). The hypoperfusion of this posterior cortical area was the major marker of symptomatic periods, as it (i) emerged during symptomatic periods in 11 patients when compared versus asymptomatic periods (right side); (ii) correlated with longer episodes in 41 patients during asymptomatic period (right side); (iii) tended to correlate with longer disease duration (right side); and (iv) strongly correlated with the severity of derealization (right and left sides).

This posterior associative cortex is involved in several functions. The angular gyrus is involved in complex cross-modal associations between somatosensory (body knowledge), auditory and visual information (Seghier, 2013). Patients with depersonalization disorder have metabolic PET abnormalities in the posterior part of the right temporal cortex (BA21 and 22) and the right angular gyrus (BA39) compared with control subjects (Simeon *et al.*, 2000). The parieto-temporal junction conveys the ability to perceive an embodied self (Corradi-Dell'acqua *et al.*, 2008) alone and in relation with the movements of surrounding people (Pavlova *et al.*, 2010). In this regard, almost all patients with Kleine-Levin syndrome reported difficulties, not only in feeling individual sensorial modalities (dull vision, impaired recognition of temperature and pain, abnormal taste), but also in integrating sensorial information (e.g. patients seeing the water flowing on their bodies but not feeling its temperature at the same time). The derealization score correlated with hypoperfusion of the right precuneus (BA7), the left inferior occipital gyrus (BA18), and not only the right but also the left angular gyrus (the largest cluster and the highest correlation coefficient). These three areas are interconnected and participate in the attention network (Seghier, 2013). The left angular gyrus is involved in left–right orientation, reading and writing, and arithmetic abilities, but these functions were not deficient (when grossly tested, no patients had Gerstmann's syndrome) in our patients during episodes, possibly because only the inferior part of the angular gyrus was affected. Why would Kleine-Levin syndrome specifically

impact the parieto-temporal junction? These structures are some of the last structures to functionally and anatomically maturate in the primate brain (Fukunishi *et al.*, 2006). As more than one-third of patients with Kleine-Levin syndrome had birth problems and developmental delays, these areas may have incompletely matured, leaving these regions more vulnerable.

In addition to derealization, all patients exhibit major apathy during an episode. Apathy is a quantitative reduction of self-generated voluntary and purposeful behaviours (Levy and Dubois, 2006). The Starkstein apathy score averaged 31/42 during episodes, which was higher than the apathy scores in several neurological and neurodegenerative diseases and shows an important auto-activation deficit in Kleine-Levin syndrome. As a comparison, apathy scores averaged 22/42 in bilateral pallidum lesions resulting in autoactivation deficits (Leu-Semenescu *et al.*, 2013), which is a syndrome considered to be a major model of apathy. Which structure or system defects could underlie the dramatic apathy in Kleine-Levin syndrome? We suspect that it is caused by the severe right medial prefrontal cortex and orbitofrontal cortex hypoperfusions during episodes. Neuropathological analyses show that these two brain areas are affected in the frontal variant of frontotemporal dementia, which includes severe apathy (Pasquier *et al.*, 1999; Rahman *et al.*, 1999), and is also affected in the frontal lesional variant of autoactivation deficit (Laplane *et al.*, 1988). Lesions of the orbitofrontal cortex (BA11) resulted in major behavioural changes in primates, including the avoidance of social contacts and isolation, as well as unusual and aberrant eating behaviours (Butter *et al.*, 1969; Myers *et al.*, 1973). In addition, most structures of the prefrontal-basal ganglia loop involved in goal-directed behaviours, including the orbitofrontal cortex, the anterior cingulum, the basal ganglia and the insula (reviewed in Levy and Dubois, 2006), are hypoperfused in patients with Kleine-Levin syndrome versus control subjects. The lack of correlation between apathy scores and specific brain areas may be a ceiling effect, because the scores approached the maximum in almost all patients.

Interestingly, Kleine-Levin syndrome was defined for a century as an intermittent hypersomnia. Some hypoperfusions in arousal systems were implicated, including the hypothalamus (which contains the histamine and hypocretin arousal systems) and the thalamus (which relays the arousal messages arising from the brainstem and hypothalamic nuclei). A clinical–imaging correlation is difficult to propose, as the hypothalamus and the thalamus were equally hypoperfused during asymptomatic and symptomatic periods, whereas patients were hypersomnolent in symptomatic periods and alert in asymptomatic periods (with notably no residual sleepiness compared to controls). We also found no correlation between sleep time during episodes or residual sleepiness and any hypoperfusion on SPECT. The brainstem, which contains most arousal systems and may be affected here, is, however, not easily visible upon scintigraphic analysis.

In conclusion, this first group analysis of brain function in Kleine-Levin syndrome highlights the dysfunction of several subcortical and associative cortical areas, as a minimum common to all patients. This dysfunction is concordant with hypersomnia, apathy and derealization, which are the most specific symptoms of Kleine-Levin syndrome. It suggests that there are long-term brain consequences in a disease that was considered before as benign and intermittent. The next step will be to determine whether subtle cognitive, psychiatric and sleep consequences may persist during interictal periods and in the long term, especially in patients with long episodes and long disease duration. Also, these brain functional abnormalities support the idea of giving a preventive treatment in patients with Kleine-Levin syndrome.

Acknowledgements

We thank Beny Charbit and Sylvie Delroise for their help in the recruiting the control subjects.

Funding

The Kleine-Levin syndrome foundation (Boston, Ma, USA) provided a grant to pay for the tests in controls. The study was also supported by the Institut National de la Santé et de la Recherche Médicale and the Assistance Publique - Hôpitaux de Paris at the Clinical Investigation Center Paris-Est (CIC-9304) of Pitié-Salpêtrière University Hospital. The collection of clinical information in patients with Kleine-Levin syndrome was part of the PHRC P070138 project (Health Ministry) coordinated by Isabelle Arnulf. The promoter of the study was Assistance Publique - Hôpitaux de Paris.

Supplementary material

Supplementary material is available at *Brain* online.

References

American Academy of Sleep Medicine. The international classification of sleep disorders—revised. Chicago, IL: American Academy of Sleep Medicine; 2005.

Arnulf I, Lin L, Gadoth N, File J, Lecendreux M, Franco P, et al. Kleine-Levin syndrome: a systematic study of 108 patients. Ann Neurol 2008; 63: 482–93.

Arnulf I, Rico T, Mignot E. Diagnosis, disease course, and management of patients with Kleine-Levin syndrome. Lancet Neurol 2012; 11: 918–28.

Arnulf I, Zeitzer JM, File J, Farber N, Mignot E. Kleine-Levin syndrome: a systematic review of 186 cases in the literature. Brain 2005; 128: 2763–76.

Billiard M, Jaussent I, Dauvilliers Y, Besset A. Recurrent hypersomnia: a review of 339 cases. Sleep Med Rev 2011; 15: 247–57.

Butter C, McDonald J, Snyder D. Orality, preference behavior, and reinforcement value of nonfood object in monkeys with orbital frontal lesions. Science 1969; 164: 1306–7.

Carpenter S, Yassa R, Ochs R. A pathologic basis for Kleine-Levin syndrome. Arch Neurol 1982; 39: 25–8.

Corradi-Dell'acqua C, Ueno K, Ogawa A, Cheng K, Rumiati RI, Iriki A. Effects of shifting perspective of the self: an fMRI study. Neuroimage 2008; 40: 1902–11.

Cox BJ, Swinson RP. Instrument to assess depersonalization-derealization in panic disorder. Depress Anxiety 2002; 15: 172–5.

Dauvilliers Y, Mayer G, Lecendreux M, Neidhart E, Peraita-Adrados R, Sonka K, et al. Kleine-Levin syndrome: an autoimmune hypothesis based on clinical and genetic analyses. Neurology 2002; 59: 1739–45.

Desgranges B, Baron JC, de la Sayette V, Petit-Taboue MC, Benali K, Landeau B, et al. The neural substrates of memory systems impairment in Alzheimer's disease. A PET study of resting brain glucose utilization. Brain 1998; 121 (Pt 4): 611–31.

Desgranges B, Baron JC, Giffard B, Chetelat G, Lalevee C, Viader F, et al. The neural basis of intrusions in free recall and cued recall: a PET study in Alzheimer's disease. Neuroimage 2002; 17: 1658–64.

Engström M, Landtblom AM, Karlsson T. Brain and effort: brain activation and effort-related working memory in healthy participants and patients with working memory deficits. Front Hum Neurosci 2013; 7: 140.

Engström M, Vigren P, Karlsson T, Landtblom A. Working memory in 8 Kleine-Levin syndrome patients: an fMRI study. Sleep 2009; 32: 681–8.

Fukunishi K, Sawada K, Kashima M, Sakata-Haga H, Fukuzaki K, Fukui Y. Development of cerebral sulci and gyri in fetuses of cynomolgus monkeys (Macaca fascicularis). Anat Embryol (Berl) 2006; 211: 757–64.

Haba-Rubio J, Prior JO, Guedj E, Tafti M, Heinzer R, Rossetti AO. Kleine-Levin syndrome: functional imaging correlates of hypersomnia and behavioral symptoms. Neurology 2012; 79: 1927–9.

Hong SB, Joo EY, Tae WS, Lee J, Han SJ, Lee HW. Episodic diencephalic hypoperfusion in Kleine-Levin syndrome. Sleep 2006; 29: 1091–3.

Huang YS, Guilleminault C, Kao PF, Liu FY. SPECT findings in the Kleine-Levin syndrome. Sleep 2005; 28: 955–60.

Huang Y, Guilleminault C, Lin K, Hwang F, Liu F, Kung Y. Relationship between Kleine-Levin Syndrome and upper respiratory Infection in Taiwan. Sleep 2012; 35: 123–9.

Johns MW. Sleepiness in different situations measured by the Epworth Sleepiness Scale. Sleep 1994; 17: 703–10.

Landtblom AM, Dige N, Schwerdt K, Safstrom P, Granerus G. Short-term memory dysfunction in Kleine-Levin syndrome. Acta Neurol Scand 2003; 108: 363–7.

Laplane D, Dubois B, Pillon B, Baulac MRNP. Perte d'auto-activation psychique et activite mentale streotypee par lesion frontale. Rev Neurol (Paris) 1988; 144: 564–70.

Leu-Semenescu S, Uguccioni G, Golmard JL, Czernecki V, Yelnik J, Dubois B, et al. Can we still dream when the mind is blank? Sleep and dream mentations in auto-activation deficit. Brain 2013; 136: 3076–84.

Levy R, Dubois B. Apathy and the functional anatomy of the prefrontal cortex-basal ganglia circuits. Cereb Cortex 2006; 16: 916–28.

Myers R, Swett C, Miller M. Loss of social group affinity fol- lowing prefrontal lesions in free-ranging macaques. Brain Res 1973; 64: 257–69.

Pasquier F, Lebert F, Lavenu I, Guillaume B. The clinical picture of frontotemporal dementia: diagnosis and follow-up.The clinical picture of frontotemporal dementia: diagnosis and follow-up. Dement Geriatr Cogn Disord 1999; 10: 10–14.

Pavlova M, Guerreschi M, Lutzenberger W, Krageloh-Mann I. Social interaction revealed by motion: dynamics of neuromagnetic gamma activity. Cereb Cortex 2010; 20: 2361–7.

Portilla P, Durand E, Chalvon A, Habert M, Navelet Y, Prigent A, et al. SPECT-identified hypoperfusion of the left temporomesial structures in a Kleine-Levin syndrome [in French]. Rev Neurol (Paris) 2002; 158: 593–5.

Rahman S, Robbins T, Sahakian B. Comparative cognitive neuropsychological studies of frontal lobe function: implications for therapeutic strategies in frontal variant frontotemporal dementia. Dement Geriatr Cogn Disord 1999; 10: 15–28.

Seghier ML. The angular gyrus: multiple functions and multiple subdivisions. Neuroscientist 2013; 19: 43–61.

Simeon D, Guralnik O, Hazlett E, Spiegel-Cohen J, Hollander E, Buchsbaum M. Feeling unreal: a PET study of depersonalization disorder. Am J Psychiatry 2000; 157: 1782–8.

Starkstein SE, Mayberg HS, Preziosi TJ, Andrezejewski P, Leiguarda R, Robinson RG. Reliability, validity, and clinical correlates of apathy in Parkinson's disease. J Neuropsychiatry Clin Neurosci 1992; 4: 134–9.

Vigren P, Tisell A, Engstrom M, Karlsson T, Leinhard Dahlqvist O, Lundberg P, et al. Low thalamic NAA-concentration corresponds to strong neural activation in working memory in Kleine-Levin syndrome. PLoS One 2013; 8: e56279.

Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983; 67: 361–70.